EXPERIMENTS ON THE EFFECTS OF INJECTION OF EGG-ALBUMEN AND SOME OTHER PROTEIDS.

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Plates XVI–XX.

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INTRODUCTION.

Although the occurrence of albuminuria after injection of certain proteids, notably egg-albumen, has long been recognized, many details of this phenomenon have received but scant attention. At the time when this research was started, in the spring of 1897, very little was known concerning the ratio of the proteid which is excreted, the time required for excretion, and the factors which influence these; nor were there any very definite data about the fate of that portion which is retained, about the changes occurring in the kidneys, and about certain toxic effects produced. Since then a number of important papers dealing with this subject have made their appearance and have in large part anticipated some of our results. However, the topic is even now far from exhausted; many questions have only been touched upon and others will bear further support. We have, therefore, extended our research along lines not originally contemplated, and the presentation of the experiments in the order in which they were performed would not do justice to the subject. We have accordingly grouped them under various headings.

The tables will take the place of more extended protocols.

TABLE I.—EXP. I–XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.

Experiment number.	Other experiments done on same animal.	Animal.		Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected.	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of proteid injected per kilo. of animal.
I	(II, III, IV).	Dog	♀	6.13	Vein.	Egg, watery, 1.148%.	1.148	0.7672	0.3825	0.187
II	(I, III, IV), (4 days since last experiment.)	"	"	"	Hypodermic.	" "	.....	Only non-coagulable proteid.	.....	.....
III	(I, II, IV), (6 days since last exp.)	"	"	"	Vein.	Normal salt, 100 cc. 4 min.	.....	.....	..... Died before excretion was completed.	.....
IV	(I, II, III), (6 days since last exp.)	"	"	"	Hypodermic.	Egg, watery, 4.156%.	2.078	1.402 +	0.338	
V	None.	"	"	5.9	Vein.	Egg, freed from globulin by dialysis, 4.470%.	2.2484	1.463	0.785	0.381
VI	"	"	"	5.3	"	Egg Syntonin in alk. solution. Proteid = 1.276%. Alkalinity = 0.376% NaOH.	1.914	Anuria to death.	... ..	0.361
VII	"	"	"	8.75	"	Alkali alb. from egg. Proteid = 0.243%; Alkal. = 0.116%.	0.243	0.0719 alkali alb. 0.0142 coagulable albumen.	.....	0.028
VIII	(X)	"	♂	6.47	Hypodermic.	Alkali alb. from egg. Proteid = 2.447%. Alkal. = 0.316%.	2.227	None.	2.227	0.344
IX	(XI)	"	♂♀	10.	Vein.	Egg, watery, 0.055%.	0.0453	0.6817	Negative.	0.0045
X	(3 days after VIII).	"	♂	6.47	Hypodermic.	" " 5.236%.	4.1364	1.3325	2.8039	0.639
XI	(4 days after IX).	"	♀	10.	Vein.	Alk. alb. from egg. Proteid = 3.073%. Alkal. = 0.4995%.	6.146	Trace, both alk. and native.	6.14	0.615
XII	None.	"	♂	5.23	Hypodermic.	Egg-albumen.	0.1218	3 days; 0 0135 4th day; 0.5861 and albumose.	.....	0.023
XII A	5 days after last.	"	"	"	"	" "	0.0609	Trace of albumose.	0.06	0.0116
XIII	None.	"	"	4.8	"	" " (free from albumose.)	1st day; 1.5266 2d " 1.6240 3d " 1.2992 6th to 9th day; 1.4078 Total, 5.8576	Little albumen, considerable albumose.	.....	1.220 in 9 days.
XIV	"	"	♀	5.7	Vein.	4.742% albumen + 0.5% Na_2CO_3 (unconverted.)	7.7303	Much albumen (urine solid on heating).	Died before complete excretion.	1.356
XV	"	"	"	2.7	"	Egg in normal salt, 1.955%.	1.7146	Much albumen.	Died before complete excretion.	0.635
XVI	"	"	"	2.7 (fat)	"	Egg, watery, 2.350%	1.892	0.7372	1.1546	0.701
XVII	"	"	"	4.55	"	Undiluted egg albumen, (14.8%.)	12.769	.....	.....	2.806
XVIII	"	"	"	4.5	"	Egg, watery, 2.849%.	2.840	1.5826	1.2574	0.631
XIX	"	"	"	4.1	"	" " "	2.849	1.5646 +	Died before complete excretion.	0.702
XX	"	"	"	7.7	"	" normal salt, 0.655%.	0.0721	0.2823 in 8 days, and still excreting.	More excreted.	0.009
XXI	"	"	"	4.8	"	" " " "	0.0629	0.1771 in 5 days.	" "	0.013
XXI A	in 6 days, 0.2 Gm. Morphine.	"	"	"	Hypodermic.	.....	.....	.....	.....	.....
XXII	None.	"	"	9.5	Vein.	Egg, watery, 1.855%	1.855	[1.4080 in 2½ hours.]	.....	0.195

TABLE I.—EXP. I-XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.—Continued.

Experiment number.	Other experiments done on same animal.	Animal.	Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of proteid injected per kilo. of animal.
XXIII A	None.	Guinea.	0.275	Hypodermic.	Egg, watery.	0.3+ in 2 days.	.....	.....	.....
B	"	"	0.245	"	" "	" " "	.....	.....	.....
XXIV A	(XXVIII).	Rabbit ♂	2.26	Ear-vein.	" "	0.156	Too small to be accurate.	. ....	0.069
B	"	" ♀	2.25	"	" "	0.281	Too small to be accurate.	.....	0.125
XXV A	None.	Guinea	0.485	Hypodermic.	" "	1st day 0.034	.....	.....	In 12 days
B	"	"	0.230			3d " 0.031	.....	.....	A 0.307
						12th " 0.084		.....	B 0.648
						Each			
						Total in 12 days 0.149			
XXVI	"	Dog ♂	5.9	Vein.	" " 3.402%	3.402	[4.662 in 1 hr. 40 min.]	.. ...	0.576
XXVII	"	" ♂	7.0	"	" " 3.4%	5.1	.....	.....	0.729
XXVIII A	(XXIV) 8 days after previous injection.	Rabbit ♂	2.21	Hypodermic.	" " 8.362%	6.469	0.963+	Died before complete excretion.	2.927
B		" ♀	2.05	Peritoneum.	" " "	5.850	0.931+		2.853
XXIX	None.	Dog ♂	5.8	Vein.	" norm. salt 3.397%	5.096	.....	.....	0.875
						A. B.			In 8 days.
XXX A	"	Guinea pig	0.33	Peritoneum.	" " " 1st day	0.203 0.203	.....	.....	A 8.119
B	"	"	0.25		4th day.	0.763 0.636			
					7th day.	0.569 0.569	.....	.....	B 10.184
					8th day.	1.138 1.138			
					Total in 8 days.	2.673 2.546			
XXXI	"	Rooster		"	" norm. salt.	0.763	None.	.....	.. ...
XXXII	"	Rabbit ♂	1.21	Vein.	" " heated to 73°, 1.255%.	1.673	[0.6174 in 1 hr.]	.....	1.382
XXXIII	"	" "	1.79	"	Human muscle in normal salt 0.389%.	0.486	None in 5 hours.	.....	0.272
XXXIV	"	Hen		"	Egg, watery.	Dies at end of injection.	... ..	.....	.....
XXXV	"	"		"	" "	Dies as wound is sewn up.	.....	.....	.....
XXXVI	"	"		Peritoneum.	" " 4.5%.	2.275	0.367	1.908	.....
XXXVII A	"	Rabbit ♀	0.975	"	" "	1st day 4.31	0.915	3.395	4.42
						5th " 3.5	0.794	2.706	3.59
B	"	" "	0.185	"	" "	1st day 4.31	1.387	2.923	3.64
						5th " 3.5	1.112	2.388	2.95
XXXVIII A	"	" ♂	1.140	"	Chicken muscle in normal salt 4.375%.	4.375 "	Died.	.....	3.83
B	"	" "	1.220	"			None.	All.	3.74
XXXIX A	"	" "	0.973	"	Dog's muscle norm. salt.	.....	None.	All.	.....
B	"	" "	0.905	"	" " " "	.....	Died.	.....	.....
XL	"	Dog ♂	3.75	Hypodermic.	Egg, watery.	0.469	None.	0.46	0.125
XLI	"	" ♀	3.	Vein.	Egg with equal vol. norm. salt.	100 cc.	Dies at end of injection.	.....	.....
XLIV	"	Rabbit	1.570	"	Dog's muscle in norm. salt.	100 cc.	None.	All.	.....

TABLE II.—CONTINUATION OF EXPERIMENTS I-XLIV. PERCENTAGES OF PROTEID RETAINED AND EXCRETED. DURATION OF EXCRETION. EXAMINATION OF URINE.

Experiment No.	Amount of proteid retained per kilo.	Per cent. of the injected proteid excreted.	Per cent. retained.	Per cent. excreted in first 24 hours.	Excretion lasted between—	Special remarks on urine.	Glycosuria.
I	0.062	66.67	33.33	.....	42 and 55 hours.	.....	1.3 gm.
II	....	....	....	.....	2 and 5 days.	Non-coagulable proteid.	None.
III	....	...	....	.....	.....	.....	1.956
IV	....	67+	....	.....	Over 3 days.	.....	None.
V	0.133	65.07	34.93	49.5	43 and 64 hours.	Small amount of globulin.	"
VII	....	....	....	.....	40 hours.	.....	"
VIII	0.344	0	100	0	0	No casts.	"
IX	....	....	....	.....	.....	Contains haemoglobin and globulin, probably from admixture of wound secretion, since this kept open.	"
X	0.433	32.	68	.....	55 and 71 hours.	No globulin.	"
XI	0.615	0	100	0	0	.....	.....
XII	0.021	11	89	.....	2 and 3 days.	No casts.	None.
XIII	....	....	....	.....	11 days after last injection.	" "	"
XIV	....	....	....	.....	.....	.....	"
XV	....	87+	....	.....	.....	.....	"
XVI	0.427	38.9	61.1	38.1	24 and 42 hours.	.....	.....
XVIII	0.279	55.7	44.3	42.	90 hours.	Trace globulin.	None.
XIX	....	55+	...	40.5	42 hours.	.....	Large amount.
XX	....	....	....	167.	10 days+.	.....	Fair amount.
XXI	....	....	....	125.	5 days.	.....	None 1st day, some 2d, none after, but plenty on morphine.
XXII	....	....	....	[75 in 2½ hours.]	.....	Much globulin.	None.
XXIV A and B	....	.. .	....	.....	.....	No albumose.	"
XXVI	...	...	....	[137 in 1 hr. 40 min.]	.....	Much globulin.	"
XXVIII A	....	14.9+	....	5.1	3 days+.	Considerable albumose.	"
" B	....	15.9+	....	13.9	3 days+.	" "	"
XXIX	....	....	....	.....	.. ..	.....	....
XXXII	....	....	...	[36.8 in 1 hour.]	.....	No globulin; no precipitate below 73°.	....
XXXIII	....	....	....	.....	.....	Proteid free.	.....
XXXVI	....	16.1	83.9	16.1	24 hours.	No globulin.	.....
XXXVII A	3.482	21.2	78.8	10.3	3 days.	Albumose increased.	.....
" "	2.775	22.7	77.3	12.7	3 days.	" "	.....
" B	2.467	32.2	67.8	17.4	2 days.	" "	.....
" "	2.015	31.8	68.2	8.1	3 days.	" "	.....
XXXVIII A and B	....	....	....	.....	.....	.....	None.
XL	0.125	0	100	0	0	No albumose.	"
XLIV	....	0	100	0	0	.....	"

TABLE III.—CONTINUATION OF EXPERIMENTS I-XLIV. SYMPTOMS AND AUTOPSIES.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
I	.....	Stupid next day, lively on 2d.	.....	Diuresis.
II	.....	None.	....	.....
III	.....	Steadily emaciating since beginning of experiment, but voracious appetite.	.....	Normal saline on sugar.
IV	3 days after last injection.	Greatly depressed next day, can scarcely walk on 3d. Temperature 39.5° C.	Extensive pyothorax; acute congestion of kidneys; heart muscle normal.	.....
V.....	Killed 5th day.	None. General condition good.	Slight cortical congestion of kidneys, particularly Malpighian tufts; cells granular.	....
VI	7 hours.	Coma and convulsions; bloody diarrhœa.	Hæmorrhage, congestion and necrosis of abdominal organs; blood changes. See p. 251.	Peculiar toxin action.
VII	.....	None.	.....	Excretion native albumen after injection of alkali albumen.
X	Killed on 3d day after last injection.	None. Good condition.	Congestion of abdominal organs, particularly cortex of kidneys.	.....
XI	Killed on 2d day after last injection.	Almost in stupor.	Kidneys mislaid; other organs practically normal.	.....
XII	Killed on 7th day after last injection.	None.	Negative.	Transitory albuminuria 4 days after injection.
XIII	About 40th day.	Emaciation.	Not made.	Long continued excretion.
XIV	4 hours.	Progressive medullary paralysis	Negative.	Death probably from embolism; injection of mixture of alkali and albumen.
XV.....	16 hours.	Not known.	Negative, but stomach distended with 10 ounces (=10% body weight) of semi-solid food.	Shock during full digestion?
XVI	.....	.....	.....	Coagulation temp. of urine.
XVII	15 minutes.	Respiratory, then cardiac paralysis.	....	Death by embolism.
XVIII	4 days.	None till last hour, then convulsive. See p. 249.	Negative. Microscopically congestion of abdominal organs.	Death under convulsions after 4 days of health.
XIX	40 hours.	Depression, anorexia. See p. 247.	Not made.	.....
XX	.....	None.	.....	Nephritic albuminuria.
XXI	1 day after morphine.	None.	.....	"
XXII	.....	.....	....	Comparison of fractional heat precipitates. Physiological effects.
XXIII A.....	28 hrs. after second injection.	None for 6 hrs., then depressed.	Much light red fluid in subcutaneous tissue and abdominal muscles. Otherwise negative; kidneys trifle congested.	Toxic effects.

TABLE III.—CONTINUATION OF EXPERIMENTS I-XLIV. SYMPTOMS AND AUTOPSIES.—Continued.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
XXIII B.....	10 hrs. after second injection.	None for 6 hrs., later not observed.	Same as XXIII A.	Toxic effects.
XXIV A & B.	.....	.....	.....	Metabolism.
XXV A	.....	Local ulcers which heal. No other effect.	.....	No toxic effect.
B	.....	None.	.. ..	" "
XXVI.....	.....	.....	....	Comparison of fractional heat precipitates. Physiological effects.
XXVII.....	.....	Complete anuria for 4 hours, although 400 cc. of liquid (=56 cc. per kg.) were injected.	.....	.....
XXVIII A & B.....	3 days.	Debility and emaciation.	....	Starvation and metabolism.
XXIX.....	.....	.....	.....	Comparison fractional heat precipitates.
XXX A & B..	About 14 hrs. after last injection.	Some depression.	Free fluid in peritoneum; else negative.	Toxic effects.
XXXI.....	....	None.	....	Rooster. None excreted.
XXXII	.....	.....	.....	Inject. of albumen heated to 73.
XXXIII.....	5 hours.	Diarrhœa, coma, fall of temperature, respiratory paralysis. See p 247.	Congestion of abdominal organs; degeneration of liver cells.	Muscle inject'n; none excreted. Toxic effects (anæsthetic).
XXXVI.....	.. ..	....	.....	Excretion egg albumen by hen.
XXXVII A & B.....	.....	None.	.....	Metabolism.
XXXVIII A.	Between 5 and 15 hours.	Not observed.	Negative.	Toxicity of chicken muscle.
B.	.....	None.	....	Retention of chicken muscle.
XXXIX A....	.....	None.	.....	Retention of dog's muscle.
B...	Between 5 and 15 hours.	Not observed.	Negative.	Toxicity of dog's muscle.
XL	.....	None.	....	Metabolism.
XLI	10 minutes.	Paralysis of respir'n and heart.	.....	Embolism.
XLIV..... ...	Killed on 3d day.	Fever.	Negative.	Myosin. Fever.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
43	Rabbit, 950 gm.	Urethane, 2.5 gm. stomach.	Intravenous injection.	Died during injection.	Acute Urethane death.	.....	.....	.....
44	Rabbit, 1570 gm.	Chloretone, 25 cc. sat. sol. stomach.	100cc. Dog's muscle solu. intravenously.	Killed with chloretone on 3d day.	Muscle and death by chloretone.	*Kidney:* normal. *Liver:* considerable fatty degeneration of epithelium. *Spleen:* normal.	*Liver:* marked in places. *Spleen:* a few pigment bearing cells.	*Liver:* none. *Spleen:* quite profuse.
45	Rabbit.	As 44.	No injection.	Died acutely.	Acute chloretone death.	*Liver:* marked congestion; cells unaltered. *Spleen:* normal.	*Spleen:* slight.	*Liver:* none.
46	Rabbit.	As 44.	As 45.	Died in 3 to 16 hours.	Subacute chloretone death.	*Kidney:* greatly congested. *Liver:* much capillary congestion. Cells generally unaltered. *Spleen:* normal.	*Liver:* some.	*Liver:* none.
47	Rabbit, ♂960 gm	Urethane, 1 gm. rectum.	Intravenous injection.	Died in 1½ hrs.	Acute urethane death.	*Kidney:* considerably congested. Cells granular. *Liver:* cells very much vacuolated and granular.	None in liver or kidney.	.....
48	Rabbit, 850 gm.	As 47.	" "	Died in 3½ hrs.	Subacute urethane death. Urine contained very many granular casts and some blood. Large quantity of free blood in abdomen; clots on removal. (Injury during autopsy ?)	*Kidney:* cells considerably degenerated. *Liver:* cells greatly vacuolated and fatty.	*Kidney:* Pigment in the epithelium of the convoluted tubules (iron free.) *Liver:* some.	*Kidney:* none.
49	Rabbit, 1310 gm.	As 47.	15 cc. 5 day brooded egg, into jugular.	Died in 5 days.	.....	*Kidney:* granular degeneration of epithelium. *Liver:* extensive capillary congestion. Thrombi in many veins and capillaries.	*Kidney:* in epithelium. *Spleen:* considerable; rather diffuse.	*Kidney:* none. *Spleen:* great amount.
50	4 guinea-pigs.	....	Each received 1 cc. of 1 day brooded egg solution subcutaneously.	One dies in 5 days.	.....	.....	.....	.....
51 A	Rabbit, 1050 gm.	Urethane, 0.75 gm. rectum.	15cc. (=0.362 gms.) of fresh egg of brooding series. *7 days later.* 11cc. (=0.254 gm.) of 6 day brooded egg into ear vein. *6 days later,* normal salt, subcutaneously.	Killed by medulla stroke, 2 days after last experiment.	.....	*Kidney:* some congestion; cells rather cloudy; no casts. *Liver;* proliferation of young connective tissue about lobules; cells granular. *Small Intestine:* slightly congested. *Cardiac muscle* and *large intestine:* normal.	*Kidney:* considerable in epithelium of convoluted tubules. *Liver:* small amount.	*Kidney* and *Liver:* none.
53 B	1070 "							
57 D	970 "							

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
51 B 54 A	Rabbit, 1000 gm. 860 gm.	Urethane, 0.75 gm. per rectum.	15 cc. (= 0.362 gm.) of fresh egg of brooding series. 7 *days later:* norm. salt, ear.	Died 7 days after last experiment.	.....	*Kidney:* considerable congestion; cells very granular. *Liver:* congested; cells somewhat cloudy. *Spleen, cardiac muscle* and *lungs:* normal. *Hyperleucocytosis* in blood of lungs and kidneys, not noticed in other situations.	Small amount in *Spleen.* None in *lungs, cardiac muscle, liver* or *kidney.*	*Spleen:* some; but most of the pigment does not take it.
51 C 53 A 56 A 57 A	Rabbit, 1000 gm. 960 " 1080 " 1050 "	Urethane, 0.5 gm. per rectum.	10cc. (=0.24 gm.) fresh egg sol. of brooding series, in ear vein. 10 *days later:* norm. salt in ear vein. 2 *days later:* 15 cc. egg soln. from 9 day brood. 4 *days later:* norm. salt intraperitoneally.	Killed by medulla stroke, 8 days after last injection.	.....	Bacterial infection profound.	*Liver:* considerable amount of yellow granules in cells. *Spleen:* large amount, granular and in lumps. *Kidney* and *cardiac muscle:* none.	*Liver:* none. *Spleen:* large amount; practically all the pigment has taken the stain.
52 A	Rabbit, 1500 gm.	Urethane, 1 gm. per rectum.	20 cc. (= 0.411 gm.) egg soln. of 3 days brood, jugular.	Died in 6 days from starvation.	.....	.....	.....	.....
52 B 55 A	Rabbit, 1030 gm. 980 gm.	Urethane, 0.5 gm. per rectum.	6 cc. (=0.123 gm.) egg soln. of 3 days brood, ear vein. 6 *days later:* 15cc. alkaline egg syntonin soln. by jugular.	Died in 2 days after last experiment.	Death from bacterial invasion.	Urine contained a few granular casts. Profound bacterial infection.	*Liver:* only in hæmorrhagic areas. *Spleen:* none.	*Spleen:* diffuse light blue stain. *Liver* or *Kidney:* none.
53 C 56 B 57 C	Rabbit, 1080 gm. 1070 gm. 1060 "	Urethane, 0.5 gm. per rectum.	15 cc. (= 0.347 gm.) egg soln. of 6 days brood, jugular. 2 *days later:* 6 cc. alkaline egg syntonin soln., ear vein. 4 *days later:* normal salt subcutaneously.	Killed by medulla stroke, 30 days after last experiment.	.....	Strong bacterial infection.	*Spleen:* considerable. *Liver:* doubtful. *Kidney* and *cardiac muscle:* none.	*Spleen:* very slight.
55 C	Dog 4.3 Kg.	Morphine. ether.	25 cc. alkaline egg syntonin soln. femoral.	Killed by heart puncture in 2 days.	.....	*Kidney:* Cells granular; great degeneration in limited areas; casts. *Liver:* slight cellular degeneration. *Suprarenal, spleen, pancreas, small intestine, and cardiac muscle:* all normal.	*Spleen:* some in granules and lumps. *Suprarenal:* some granules in cortex, normal pigmented cells in medulla. *Liver, kidney,* all kinds of *muscle:* none.	*Spleen:* considerable. *Kidney* or *intestine:* none. *Liver:* diffuse light stain of cells, but granules only in thrombus.
55 B 57 B	Rabbit, 1090 gm. 1090 gm.	....	10 cc. egg soln. of 9 days brood, ear vein. 4 *days later:* norm. salt intraperitoneally.	Died 16 days after last experiment.	.....	Strong bacterial infection.	*Spleen:* considerable. *Liver:* some. *Kidney* and *cardiac muscle:* none.	*Spleen:* very slight.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
60	Rabbit, 785 gm.	....	6 cc. norm. salt, in ear vein.	Killed by medulla stroke in 3 days.	.....	Slight bacterial infection.	*Kidney* or *liver*: none. *Spleen*: considerable amount, granular and clumps.	*Spleen*: very slight and diffuse.
61	Rabbit, 834 gm.	....	8 cc. norm. salt, in ear vein.	As 60.	.....	As 60.	Little in *spleen* or *suprarenal*; a few granules in *liver-cells*. None in *kidney*.	*Spleen*: very little. *Liver*: none.
62	Rabbit, 885 gm.	Urethane, 0.5 gm, per rectum.	25 cc. alkaline egg syntonin into jugular.	Killed by medulla stroke in 10 days,	Long fever.	*Kidney*: Rather slight granular swelling of epithelium, considerable congestion of capillaries and tufts. *Liver*: considerable capillary congestion; cells normal. *Spleen* and *suprarenal* normal	*Spleen*: considerable, granular and in lumps. *Liver*: ditto: *Kidney*: none.	*Spleen*: very large amount. *Liver*: none.
63	Rabbit, 1180 gm.	....	1 cc. alkaline egg syntonin in ear vein.	Killed by medulla stroke in 3 days.	.....	Moderate bacterial infection.	*Spleen*: considerable, *Liver*: rather large amount of granular.	*Spleen*: considerable. *Liver*: none.
64	Rabbit, 1195 gm.	Urethane, 0.5 gm. per rectum.	Died on table.	....	.....	Strong bacterial infection.	*Spleen*: little. *Liver*: much, granular.	*Spleen*: weak, diffuse. *Liver*: a little in spots, diffuse and coarsely granular.
65	Rabbit, 1225 gm.	As 64.	20 cc. alkaline egg syntonin, jugular.	As 63.	.....	Very slight bacterial infection: *Kidneys*: hyaline degeneration in cells of convoluted tubules. *Liver*: diffuse granular degeneration of cells. *Spleen*: normal.	*Kidney*: many granules in epithelium. *Liver*: considerable free granules. *Spleen*: small.	*Liver*: none. *Spleen*: considerable.
65 A	Dog.	Morphine-ether.	50 cc. alkaline egg syntonin, femoral.	Killed in 3 days.	.....	Congestion of *abdominal viscera*. *Small intestine*: degeneration of epithelium of villi. *Kidney*: extensive hyaline degeneration and cloudy swelling. *Liver*: epithelium normal, also *suprarenals* and *large intestine*.	*Spleen*: considerable, in clumps. *Intestine*, *liver* and *kidney*: none. *Suprarenal cortex*: slight.	*Spleen*: considerable. *Liver*: none. *Kidney*: some in isolated places in cells.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
66	Rabbit, 1255 gm.	....	2 cc. sol. of rabbit's muscle, in ear.	Killed by medulla stroke in 3 days.	Few bacteria.	*Kidney:* Cells as in 65, but less. *Liver:* considerable cellular infiltration of perilobular tissue. Some congestion, especially of central veins. Blood appears to contain large number of leucocytes. Cells normal. *Spleen* and *pancreas:* normal.	*Spleen:* small amount. *Liver* and *kidney:* considerable.	*Spleen:* small amount. *Liver* and *kidney:* none.
67	Rabbit, 1380 gm.	....	1 cc. as 66.	As 66.	.....	*Kidney, liver, spleen* and *suprarenal* are all normal.	*Spleen:* very little. *Liver:* some granules in cells. *Kidney* and cortex of *suprarenals:* none.	*Spleen:* almost none. *Liver:* none.
68	Rabbit, 1375 gm.	0.5 gm. urethane, per rectum.	25 cc. soln. of rabbit's muscle, jugular.	As 66.	.....	*Kidney:* extensive cloudy swelling of epithelium of convoluted tubules. Hyaline casts. *Liver:* slightly fatty, otherwise normal. *Spleen* and *Suprarenal:* normal.	*Spleen* and *liver:* considerably granular. *Suprarenal* and *kidney:* none.	*Spleen:* considerable. *Liver:* none.
69	Rabbit, 1435 gm.	As 68.	As 68.	Killed by medulla stroke 10 days.	.....	*Kidney:* some cloudy swelling and vacuolization of epithelium. *Liver, spleen, suprarenal:* normal.	*Spleen:* very little. *Liver:* considerably granular in cells. *Suprarenal cortex:* none.	*Liver:* and *spleen:* none.
70	Rabbit.	....	.....	Killed by medulla stroke.	Normal.	*Kidney:* considerable cloudy swelling of the epithelium of the convoluted tubules. *Liver:* slight congestion, with extensive granular degeneration of cells.	*Spleen:* almost none. *Liver* and *Kidney:* none.	*Spleen:* almost none.
71	Rabbit, 730 gm.	0.5 gm. urethane by rectum.	.....	Killed by medulla stroke in 4 days.	Urethane alone.	*Kidney:* very slight degeneration and vacuolization. *Liver:* slight capillary congestion. Cells normal.	*Kidney:* none. *Liver:* slight.	*Liver:* none.
72	Rabbit, 870 gm.	As 71.	.....	Killed by medulla stroke in 7 hours.	As 71.	*Kidney:* some cloudy swelling. *Liver:* extensive granular degeneration, as 70. *Large intestine:* normal.	*Spleen:* considerable. *Kidney* and *liver:* none.	*Spleen:* considerable diffuse pigmentation.

One of us (Sollmann) is responsible for experiments I to XXI, and 44 to 72. The others were done by us jointly. We take this opportunity of acknowledging our obligations to Professor G. N. Stewart, who first directed our attention to this subject, and in whose laboratory the first experiments were made. We are also greatly indebted to Professor Wm. T. Howard, Jr., for the preparation and description of the greater part of the histological material.

II. METHODS.

A. Operation.

A known quantity of egg-albumen, obtained by dissolving egg white in distilled water or normal salt solution (and always containing a small quantity of egg-globulin), was injected under anæsthesia into the femoral vein (dog) or into the jugular (rabbit); or without anæsthesia into the ear vein or intraperitoneally or hypodermatically, and the urine was collected by the methods presently to be described. The dogs were anæsthetized with morphine and ether, using the smallest quantity of the latter which would keep the animal quiet. To insure against albuminuria from the anæsthetic, the urine was drawn and analyzed immediately before injection. The absence of a notable quantity of globulin in typical experiments is also an indication that in these no part of the albuminuria resulted from the anæsthetic. The anæsthesia was performed on rabbits by injecting through a catheter a solution of chloretone into the stomach, or urethane into the rectum. The amounts are noted in the foregoing tables. The injection of the proteid solution was made fairly rapidly through a burette connected with the femoral vein by a glass cannula. It was aimed to make the operation aseptic, but in the case of the dogs we were not uniformly successful in this respect.

The method of collecting the urine differed with the experiment. After intravenous and intraperitoneal injection, the animals were placed in a zinc-lined or galvanized-iron cage, with an opening for the collection of the urine. The cage was washed daily with distilled water and the washings placed with the urine; it was then flushed with a small quantity of saturated boric acid solution, and some of this was also placed in the collecting vessel. The urine collected in this way showed no trace of decomposition. In the metabolism experiments, the animals were catheterized daily and their bladders rinsed with distilled water. These animals were fed purely on oats and water. The dogs received their customary diet of meat, bread and water.

With intravenous injection, the dogs (female) were catheterized before the injection was made, and the catheter was left in position for an hour or two after the injection; the animals being kept on the operating board, lightly under the influence of ether. The wound was then stitched and covered with collodion and the animals were placed in cages for the further collection of urine. In the experiments in which it was desired to know only the ratio of the excretion of the different proteids, thin glass tubes were tied into the ureters and the urine was collected directly from these, the animal in this case not being allowed to recover from the anæsthetic.

The same animals were sometimes used a second time after they had completely recovered from the effect of the first injection. When an animal died, a careful autopsy was made and the kidneys were examined histologically after hardening and sometimes also in the fresh condition. In certain cases, other organs were also preserved in Orth's fluid, sectioned, and stained with hæmatoxylin, eosin, and for iron.

B. Analytical Methods.

In a majority of the experiments we directed our attention mainly to the total quantity of proteids, but tested in each of experiments I-XLIV for sugar (Trommer's test) and for non-coagulable proteid (ferrocyanide reaction in the absence of coagulable proteid). For further analysis the urine, if not already acid, was treated with 1% acetic acid until it just reddened litmus paper. It was then filtered and the coagulable proteid determined gravimetrically in a definite volume (10-100 cc.). The same method was used for the albumen solution. The urines of experiments I-XLIV were always tested qualitatively and often quantitatively for globulins by Pohl's method (half-saturated sol. ammonium sulphate).

In the experiments in which we intended to weigh separately the precipitates occurring at different temperatures, we heated the urines slowly in a large water-bath and kept them at the desired temperature for ½ hour. If the quantity of material admitted, a separate sample was taken for each temperature. In the observations on metabolism, where we wished to determine the quantity of the various nitrogenous constituents, the small amount of material and the numerous determinations to be made rendered it necessary to institute some preliminary experiments for the selection of a suitable method of analysis.

Preliminary Experiments for the Selection of a Method of Analysis.

1. *Determination of Urea.*—The separation of the different nitrogenous constituents was effected in the first place by phosphotungstic acid in hy-

drochloric solution...As has been shown by various experimenters, the precipitation of nitrogenous bodies by this substance is not perfectly rigorous. The amount of precipitation varies with the time during which the reagent is allowed to act, with the sample of the reagent and with the dilution. The following results are given by the different observers who have investigated this subject. Our own observations are included in this compilation:

TABLE V.—PRECIPITATION OF NITROGENOUS SUBSTANCES BY PHOSPHOTUNGSTIC ACID.

[Where a space is left blank in the table, the substance has not been investigated].

	Mallet (1).*	Reid (2).*	Schöndorff (3).*	Sollmann and Brown.
Precipitated--				
Egg albumen	Precipitate, insoluble in hot water.	.....	.....	Prec. complete.
Serum proteid	"	.....		
Myosin	"		Prec. complete.	
Vitellin	"			
Syntonin	"			
Casein	"			
Legumin	"			
Fibrin	"			
Haemoglobin	"			
Albumoses	"	80 to 90% prec.		Prec. complete.
Gelatin	"		.....	
Chondrin	"			
Xanthin series	.....	Precipitate.		
Xanthin	.....	.....		Prec. complete.
Uric acid	.....	.....	Prec. complete.	" "
Caffein	.....	.....	" "	
Guanin	.....	.....	" "	
Lysin	.....	"	" "	
Creatinin	"	"		
Ammon. sulphate	.....	.....	" "	Precipitate 87%
			.....	Used Schuchardt's acid. Kahlbaum's precipitates only partially, Merck's completely (Pfandler).
Creatin	Prec. soluble in hot water.	0	0	
Peptone	"			
Glutamin	"			
Betain	"			
Hypoxanthin	"			
Carnin				
Not precipitated—				
Urea	"	0	0 (absolutely)	0 (absolutely)
Alanin	0	.....	0	
Allantoin	0	.....	0	
Alloxantin	.....	.....	0	
Asparagin	0			
Aspartic acid	0	0	0	
Glycocoll	.....	.....	0	
Glycosin	0	0		
Glutamic acid	0	0		
Leucin	0	0	0	
Sarcosin	.....	.....	0	
Taurin	.....	.....	0	
Tyrosin	0	0	0	

*These numbers refer to the bibliography at the end of this article.

Phosphotungstic acid allows us to separate therefore:

In solution: Urea, amido-acids and peptones. Since the last two exist only in traces, the filtrate may practically be interpreted as urea.

In precipitate: Proteids (coagulable and albumoses), uric acid, xanthin bodies and ammonia.

The following protocols give the experiments made to test the method. The detail of the tests are the same as given below (p. 222).

1. Phosphotungstic acid + egg-albumen = Precipitate (filtrate free from biuret.)
   " " + Peptone (Witte) = Precipitate.
   " " + Sodium Urate = "
   " " + Ammon. Sulphate = "
   " " + Urea = no precipitate.
2. Urea: the filtrate yields 87% of the calculated nitrogen.
3. Ammonium Sulphate: the filtrate yields 13% of the calculated nitrogen of the ammonia.
4. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 104% of the urea.
5. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 114% of the urea.

It will be seen that the figures for the urea are somewhat in excess.

2. *Determination of Ammonia.*—This was done by Schloesing's Method, which was tested as follows:

1. Parallel determinations on ammon. sulphate solutions give:

Desiccation	1.000
Distillation with sodium hydrate	0.925
Sulphuric acid by barium	0.977
Schloesing	0.905
Hypobromite	0.778 *

2. Schloesing's method on a mixture of ammon. sulphate, urea, sodium urate, peptone (Witte), and coagulable proteid yields 103% of the ammonia.
3. Schloesing's method on another similar mixture yields 107% of the ammonia.

The method gives slightly excessive results.

3. *Determination of Coagulable Proteids.*—This was done by heating the slightly acidulated urine with sodium sulphate to boiling, and then adding a few drops of ferric acetate. A Kjeldahl determination was then done on the precipitate and filter, the nitrogen of the filter being subtracted.

Ferric acetate gives no precipitate with sodium urate or Witte's peptone.

The difference obtained by subtracting the nitrogen of the urea, ammonia and coagulable proteid from the total nitrogen represents the nitrogen of albumoses, xanthin bodies and uric acid, minus the somewhat variable factor of error in the above determinations.

To eliminate this uncertain quantity and to obtain a further insight into the make-up of this rest we tried various methods of directly esti-

*The decomposition with hypobromite was undertaken with a view of utilizing this method for the determination of the ammonia. It is seen that the result is unreliable.

mating these constituents; but, it must be confessed, with indifferent success.

Our experiments were restricted to such quantities and concentrations as we could expect in rabbit's urine, and we cannot make any statements concerning the usefulness of these methods when employed under other conditions.

4. *Determination of Albumoses.*—We tried the bromine and zinc-sulphate methods (for details see below). The following are our results:

(*a*) *Bromine Method* (Wiley) (4): 1. Mixture of Witte's peptone and uric acid yields 74 per cent of the albumose.

2. Mixture of Witte's peptone, egg-albumen, sodium urate, urea and ammonium sulphate yields 79 per cent of the albumose.

Whilst the method is inaccurate, the comparative values obtained by it are probably useful.

b) *Zinc-Sulphate Method.*—This was tried only in the last series of experiments, but gave the most promising results of all:

1. Peptone (Witte): Completely precipitated; no biuret in filtrate.
   Egg-albumen: Completely precipitated; no biuret in filtrate.
2. Sodium urate, Urea, } no precipitate.

(*c*) *Almen's tannin mixture* gave the following results:

1. Sodium urate: Precipitated 71 per cent of the uric acid.
   Ammonium sulphate and urea: No precipitate.
2. Sodium urate, Peptone (Witte), Egg-albumen, } were mixed with urea and ammonium sulphate. The precipitate with Almen's mixture contained but 30 per cent of their nitrogen.

This reagent is therefore not applicable.

The methods of directly estimating the alloxur bodies and uric acid appear to require much larger quantities of urine than we had at our disposal, and demand so much time that we did not give them a thorough trial.

The *methods* finally adopted are the following:

1. *Total nitrogen,* by Kjeldahl: 2 to 5 cc. of urine are placed in a 250 cc. flask with 20 cc. of concentrated sulphuric acid, mercury being added in just sufficient amount to aid oxidation, and the mixture boiled until almost colorless. The flask is allowed to cool and the contents rinsed into a litre flask with three portions of distilled water, of about 75 cc. each. A few pieces of metallic granulated zinc are then added, followed quickly by 70 cc. of a 40% (by weight) solution of caustic soda and 10 cc. of 40% solution of sulphuret of potash. The flask is quickly connected with a Liebig condensor, and heated until the distillate is free from ammonia. The distillate is received in a flask fitted air tight to the condensor. This flask contains a measured amount of decinormal sulphuric acid, and is also connected with a U-shaped absorption tube charged with the same. The distillate is then titrated, methyl orange (U. S. P.) being used as an indicator.

A blank determination was made on every new lot of chemicals and corrections applied if necessary.

Duplicate determinations were made in many cases, but not in all.

2. *Phosphotungstic Filtrate.*—A solution of phosphotungstic acid (100 ccm. phosphotungstic acid (Schuchard) to 800 cc. of 4% hydrochloric acid) is added to 5-20 cc. of urine diluted to about 30 cc., until no further precipitate occurs, then made up to 50 cc. and allowed to stand from one to three hours, filtered, and the nitrogen determined as above on 20 or 25 cc. of the filtrate. The result gives the nitrogen of *urea*.

3. *Ammonia* (Schloesing).—20-50 cc. of urine are mixed with 10-20 cc. of milk of lime and placed in a desiccator with a porcelain capsule which contains 10-20 cc. of decinormal sulphuric acid. This is allowed to stand for five days and the excess of acid titrated. The result gives nitrogen of *ammonia*.

4. *Coagulable Proteids.*—50-150 cc. of urine are rendered faintly acid with acetic acid, mixed with an equal volume of 10% sodium sulphate and boiled. When near the boiling point a few drops of ferric acetate are added. This is filtered, the precipitate washed until free from sulphates, and the nitrogen determined in the filter and precipitate. The amount of nitrogen contained in the filter is determined once for all and subtracted. This procedure was used only for the urines collected after injection.

5. *Albumoses.—a. Bromine method.*—25 cc. of urine are rendered strongly acid by concentrated hydrochloric acid, 2 cc. of bromine are added, the mixture is shaken, stoppered tightly, and left to stand over night. Some undissolved bromine must remain in the flask. The mixture is then filtered, and the precipitate washed by decantation. The filter is then returned to the flask and subjected to a Kjeldahl determination. Subtraction of the nitrogen of the filter gives the nitrogen of the total proteids. Subtraction from this of the nitrogen of the coagulable proteids leaves that of non-coagulable proteids.

b. Zinc-sulphate method.—25 cc. of urine are saturated with 35 grm. of crystalline zinc sulphate,* and filtered; the precipitate is washed with saturated solution of zinc sulphate and subjected to Kjeldahl, as in (*a*).†

* It is necessary to use tested zinc sulphate. One commercial sample yielded 2.1 mg. nitrogen per grm., or in a test carried out as above, about 1 mg. for the 25 cc. of urine.

† According to Bömer, it would have been better to acidulate the urine, and the saturated zinc sulphate solution with 2 cc. per 100 cc. of 1:5 sulphuric acid.

By these methods we could determine the following data:

(a) *Total nitrogen*—Method 1.—Exact.

(b) *Urea nitrogen*—Method 2.—Somewhat in excess.

(c) *Non-urea nitrogen*—(a minus b).—Too low.

(d) $\frac{\textit{Urea nitrogen}}{\textit{Non-urea nitrogen}} = \frac{b}{c}$—Somewhat in excess.

(e) *Ammonia*—Method 3.—Slight excess.

(f) *Coagulable proteids*—Method 4.—If anything, slight excess.

(g) *Non-coagulable proteids*—Method 5 minus (f).

(h) *Alloxur and uric acid* = a—(b + e + f + g).—Open to too many sources of error to be very reliable, but still of limited value.

III. EXCRETION OF THE PROTEID.

We shall discuss this subject under the following headings:

A. Proportion of the coagulable proteid excreted.

B. What determines the quantity of albumen retained.

C. Nature of the excreted proteid.

D. Excretion of the different constituents of egg-albumen.

E. Duration of the albuminuria.

F. Beginning of the excretion of albumen.

G. Relative quantity excreted in successive periods.

H. Retention of other proteids after intravenous or subcutaneous injection.

A. Proportion of the Coagulable Proteid Excreted.

Older observers, working mainly with qualitative methods, claim that injected egg-albumen is excreted unchanged, almost in its entirety. Thus Stokvis (5) states that egg-albumen given hypodermically "stellt eine durchaus unbrauchbare Substanz dar." But all later experimenters who have investigated this subject quantitatively, agree that this excretion is generally far from complete.

Observations on the quantitative excretion of proteid indicate the need of special precautions to guard against nephritic albuminuria. We have encountered this condition not infrequently. Excluding, therefore, all cases in which the amount excreted exceeded that injected; those in which the albuminuria continued indefinitely; those in which the urine contained considerable globulin; those in which it showed blood-pigment; and finally also those in which the animal died

before the excretion had ceased, we can present the following results bearing on this question:

After hypodermic injection of very small quantities (the largest 0.125 grm. per kilo. body weight) into either dogs or rabbits, none of the proteid is excreted:

Dogs, hypodermic injection, 0.02 to 0.43 grm. per kg., two cases, retained 68 and 89%.

Dogs, intravenous injection, 0.19 to 0.7 grm. per kg., four cases, retained 33 to 61%; mean, 40%.

Rabbits, intraperitoneal injection, 3 to 4.4 grm. per kg., four cases, retained 66 to 80%; mean, 72%.

The following cases were found in the literature:

Forster (6): Dog, intravenous, large quantity, retained 27.4%.

Lehmann (7): Dog, intravenous, small quantity, retained 23%.

Munk and Lewandowsky (8): Dog, slow intravenous, very small quantity, retained 82%; rabbit, slow intravenous, retained 54%; rabbit, intraperitoneal, retained 68%.

Adding these to ours, we see that, when moderate to large quantities are injected, the *retention* is as follows:

	Dog, intravenous.	Dog, hypodermic.	Rabbit, intravenous.	Rabbit, intraperitoneal.
Number of cases	7	2	1	5
Extremes	23 to 82%	68 to 89%	54%	66 to 80%
Means	35%	78%	....	71%

B. What Determines the quantity of Albumen retained?

From the results given in the preceding section, it is seen that the retention varies from 23 to 100% of the amount injected. It is likewise evident that:

1. Very small quantities are retained completely, at least when given hypodermically.

2. With very large quantities (Forster's case) a larger absolute amount is retained, but the proportion of that retained to that excreted is smaller.

3. The greatest retention occurs on hypodermic injection. Less is retained on intraperitoneal, and least on intravenous injection.

From 3 we may conclude that the *retention varies with the slowness of absorption*, and from 1 and 2 that *the absolute amount retained varies as the quantity injected;* whilst the *proportion retained is inverse to the quantity injected.* A more minute analysis also shows

that, while this is the general tendency, the proportion retained is less readily influenced by the injected quantity, than is the absolute amount retained, differences of 50% in the injection altering the proportion excreted but very little.

A relation can also be made out between the retention and the rapidity and duration of the excretion of albumen. Retention varies with the length of time required for excretion, but inversely to the quantity excreted in the first 24 hours.

Evidently the less the amount of albumen introduced and the slower its absorption and excretion, the more thoroughly is it retained. It may be assumed that the capacity of the organism for its utilization is limited, and these conditions would be the most favorable to it.

However, certain unknown factors also exist, as might be expected. Thus, in experiments XVI and XVIII the same amount was injected in the same manner: One animal retained 44%, the other 61%. But such instances are surprisingly few.

C. Nature of the Excreted Proteid.

The identity of the excreted with the injected proteid has been amply demonstrated for various substances (Munk and Lewandowsky). Stokvis showed by polarimetric methods that this holds true for egg-albumen. We thought it interesting to compare the *fractional coagulation temperatures* of the injected and excreted proteids and found the closest agreement. We often encountered some globulin in the urines; its quantity was ordinarily very small, and could be referred to the traces existing in egg-white. In certain cases a considerable quantity was excreted, but in these there was usually some other indication of nephritic changes.

D. Excretion of the different Constituents of Egg-Albumen.

The hypothesis has been repeatedly advanced that the precipitates which occur in solutions of egg-albumen at 57.5; 67; 72; 76; and 82° C. represent so many distinct chemical entities. Since we have in the kidney an instrument which is evidently capable of separating with great nicety very closely allied proteids, the thought suggested itself that the partial retention of egg-albumen was perhaps due to the complete retention of certain of these constituents, with

complete excretion of others. To solve this problem, we determined quantitatively the ratio of the precipitates at different temperatures in the injected solution and in the urine.

To secure strictly comparable results, both were largely diluted with water. To make the salt concentration the same in both the injected solution and the urine, the latter (previously boiled and filtered) was added to the albumen solution in the same quantity as was used for the estimation of the proteids in the urine itself. The acidity was also carefully brought to the same degree in both. The injection was always made intravenously.

Table VI gives a compilation of the ratios:

TABLE VI.

Ratio of Proteids Precipitating at Different Temperatures.

	Injection.		Urine.		Serum.	
Degrees Centigrade	68 : 70 : 73 : 77		68 : 70 : 73 : 77		68 : 70 : 73 : 77	
Experiment XXII..	36.4 : 15.2		56.6 : 11.1		.....	.....
	51.6	48.4	67.7	38.3		
Experiment XXVI .	31.8 : 0	19.4 : 50.9	27.3 : 16.2	15.8 : 40.7		... .
	31.8	70.3	43.5	56.5	92.5	7.5
Experiment XXIX .	29.7 : 9.8	15.6 : 45.6		29.1 : 60.3	86.5 : 8	0 : 5.6
	39.5	61.2	10.6	89.4	94.3	5.6

The figures give the percentage of the total proteid, precipitated at the given temperature.

It is seen that in experiment XXIX, where there was no admixture of globulins, and where serum-proteids can therefore probably be excluded, the lower proteids (68° and 70°) are lessened, *i. e.*, they are retained to a greater extent, or else their coagulation temperature is raised.

Experiments XXII and XXVI, on the other hand, show a relative increase in the lower proteids; but since these contained a large amount of globulins there was an undoubted excretion of serum, and since the latter contains almost purely the lower members, the result is not surprising.

If we calculate the quantity of serum in these urines on the basis, that the serum-albumin amounts to one half of the globulin,—certainly

the smallest possible amount *—and subtract the serum proteids calculated on this basis from the total proteids of the urine, these experiments will also bear out the conclusions formulated from experiment XXIX, the ratios now standing:

	Solution injected. 70° : 77°	Urine. 70° : 77°
Experiment XXII.....	51.6 : 48.4%	34 : 61%
Experiment XXVI.....	31.8 : 70.3%	17.9 : 82.1%

As a converse of this, we investigated in experiment XXX the unabsorbed liquid remaining in the abdominal cavity of a guinea-pig which had died between 3 and 14 hours after an intraperitoneal injection.

	68°	70°	73°	77°
Albumen injected	37.6	25.8	12.	26. %
Peritoneal liquid	53.	13.	21.	12. %

There is a greatly increased proportion of the lower proteid. We did not investigate whether this was due to admixture of serum, or to slower absorption of the lower portion of the egg-albumen.

It having been proved by the above experiments that *the lower proteids are either retained in larger proportion or changed into those of higher coagulation temperature*, we tried whether the higher proteids would be converted into the lower. For this purpose we injected an egg-solution which had been kept at 73° C. for 10 minutes. The urine gave *no precipitate up to 73°*, but a copious precipitation occurred above this temperature. 1.673 grm. had been injected, 0.617 was recovered in one hour.

We consider that this fact lends support to the view that the precipitates occurring in egg-albumen at different temperatures really belong to different proteids; the lower being more completely retained by the body, or converted into proteids of higher coagulation temperature; whilst the higher are less completely retained, and cannot be converted into the lower.

Friedenthal and Lewandowsky (10) have shown that serum loses its toxicity by heating to beginning turbidity (55 to 60°). The above experiments, in showing that excretion occurs when the solu-

* Cloetta (9) finds in acute nephritis in rabbits produced by aloin that there are 2.37 to 3.71 times as much albumin as globulin in the urine.

tion has been heated to 73°, prove that the non-retention of egg-albumen cannot be due to its content of some such toxin.

In this connection we investigated whether the kidneys of *chicken* also recognized ovalbumin as a foreign proteid.

Since the animals frequently died during or shortly after intravenous injection, the intraperitoneal method was resorted to. The fæces were extracted with water, and this extract tested for proteids in the usual manner.

Exp. XXXI.—Rooster.—Injected solution containing 0.763 grm. egg-albumen. None recovered on two days following.

Exp. XXXVI.—Hen.—Injected 2.275 grm. egg-albumen in solution. On following day the fæces yielded no globulin, but albumen as follows:

68°	70°	73°	77°	total
none	0.1305	0.1230	0.1135	0.3670

The chicken therefore behaves towards the injection of egg-albumen precisely like mammals; the retention of the lower proteids is still more complete.

E. Duration of the Albuminuria.

In experiments in which no nephritis existed, the albumen disappeared from the urine in:

				Mean.
Dogs.	Intravenous injection	(4 cases),	$1\frac{1}{2}$ to $2\frac{1}{2}$ days.	2 days.
"	Hypodermic "	(2 "),	2 to $3\frac{1}{2}$ "	3 "
Rabbits.	" "	(1 "),	3 "	
"	Intraperitoneal "	(5 "),	2 to 3 "	$2\frac{1}{2}$ "

In 4 experiments on dogs in which nephritis developed (as shown by the excretion of a larger amount of proteid than that injected), the albuminuria lasted from 3 days to several weeks, *i. e.* as long as the animal was kept under observation. Two dogs which excreted non-coagulable proteid after hypodermic injection showed this from 4 to 11 days.

It appears from the above that albuminuria in typical experiments lasts from $1\frac{1}{2}$ to 3 days, according to the manner of introduction. The comparatively long duration after hypodermic and intraperitoneal injection may be referred in large part to slow absorption of the solution.

A large lump persisted for two days at the site of hypodermic injections in rabbits; and, in cases of intraperitoneal injection, proving fatal in three hours or more, much liquid with white flakes was found in the abdominal cavity, although no other sign of inflammation was perceptible.

R. Winternitz (38) found that, after the injection of sterile egg-albumen into the pleural cavity of dogs, the amount of liquid in the pleura had considerably increased beyond the injected quantity in six hours; in sixteen hours, in one dog, it had only fallen to one-half; in another dog there was a trifle more than the injected amount after seventeen hours. The pleural liquid in all three animals contained flakes of coagulated proteid.

The excessively long persistence of a slight albuminuria in a few cases need not necessarily be referred to the injection. Haack (20) claims that the dog's urine frequently contains some albumin. M. Kaufmann (21) quotes F. N. Schulz, that rabbits show a light degree of albuminuria from the most insignificant causes; and Kóssa (23) claims that an idiopathic albuminuria is so frequent that of the numerous rabbits bought in half a year for the physiological institute in Budapest, scarcely two or three were free from it. Others, obtained from a different source, developed it after being caged for a few weeks. The rabbits used by us for quantitative experiments were always free from any noticeable albuminuria.

Our results bear out the statements of other observers on the excretion of egg-albumen. Munk and Lewandowsky (8), after injections into the ear-vein of rabbits, found the albumen mostly excreted on the 2nd day, with perhaps a trace on the 3rd. O. Weiss (11) also found quick excretion after intravenous injection of serum into the rabbit; but on subcutaneous injections he sometimes saw it last for weeks.

Forster (6), using much larger quantities, found all excreted on the 3rd day. Lehmann (7) found the excretion almost complete in one day, except in one case in which there was nephritis.

F. Beginning of the Excretion of Albumen.

The excretion always began quite rapidly. In Experiment I, in which particular attention was directed to this point, traces were made out in seven minutes after injection; the quantity increased gradually, until in 22 minutes the urine became solid on heating.

TABLE VI.—PERCENTAGE OF THE INJECTED ALBUMEN EXCRETED ON SUCCESSIVE DAYS.

	Dogs. Intravenous.						Dog. Hypodermic.	Rabbit. Hypodermic.	Rabbit. Intraperitoneal.				
Experiment and amount injected...	XIX. 2.849	XVIII. 2.840	V. 2.248	XVI. 1.892	Forster. 73.3	IX. .0453	X. 4.1364	XXVIII A. 6.469	XXVIII B. 5.850	XXXVII A. 4.313	XXXVII A1. 3.493	XXXVII B. 4.313	XXXVII B1. 3.493
First 24 hours	17 hours. 33⅓%	18 hours. 38.4%	23 hours. 49.5%	38.1%	62%	48 hours. 13 times.	55 hours.	5.1	13.9	10.3	12.7	17.4	8.1
Second 24 hours.....	17 to 41 hrs. 21.6%	18 to 42 hrs. 14.5%	23 to 43 hrs. 13.9%	24 to 42 hrs. 0.9%	8.4%		32%	4.6	14.2	10.3	6.8	13.9	10.4
Third 24 hours	Dead.	42 to 67 hrs. 2.8%	43 to 64 hrs. 1.6%	None.	2 %	.....	None.	5.1	5.3	1.0	3.0	0.8	7.3
Fourth 24 hours.....	.....	Trace.	None.	.....	.....	.....	.....	Dead.	Dead.	0.5	None.	None.	None.

It is seen in Table VI that when the injection is *intravenous* (4 experiments), *about ¾ of the excretion takes place inside of the first 17 hours; the remainder in the next 15 hours, and only traces later.* Where hypodermic and intraperitoneal injections are made, the excretion is more nearly equal on 3 successive days, or may even be higher on the second day than the first. This may be explained by slow absorption.

As to the *quantity excreted in the first few hours,* this must be largely influenced by diuresis—an extremely variable factor.

In one experiment (XXXII) of intravenous injection in a rabbit, in which the animal received 1.663 grm., 37% of the total amount was excreted in one hour. This certainly shows that the process of excretion is a very rapid one.

H. Retention of other Proteids after Intravenous or Subcutaneous Injection.

The recent work of Munk and Lewandowsky (8) has demonstrated the practically complete retention of casein and nucleo-proteid, and the retention of 85% of gelatine. Schaefer and collaborators (22) have shown that considerable proportions of proteoses and similar substances are retained. The complete assimilation of serum-proteids—whether from the same or from foreign species—is almost universally accepted. The common statement is that *alkali-albumin* is also completely retained. We made some experiments on this point.

Exp. VI.—Animal died under anuria.
Exp. VII.—Intravenous, 0.028 grm. per kg. Animal excreted both native and alkali-albumin. Probably some nephritis.
Exp. VIII.—Hypodermic, 0.344 grm. per kg. Urine proteid-free.
Exp. XI.—Intravenous, 0.615 grm. per kg. Urine proteid-free.

Our results therefore agree with those of other observers. We further determined that actual conversion was necessary, for a simple solution of egg in 0.5% sodium carbonate caused the appearance of a large amount of native albumen in the urine (experiment IV).

The statement of the text-books is that *myosin* is not excreted. This appears to rest entirely on an experiment of Lehmann (7) made with frog's myosin. In six experiments we injected extracts of

human, rabbit's, dog's and chicken's muscle into rabbits, intravenously or into the peritoneum. In no case was there any excretion. *The myosin of foreign species is, therefore, also completely retained.*

IV. EFFECTS UPON METABOLISM.

From the experiments detailed in the preceding section, it results beyond doubt that a considerable proportion of the injected egg-albumen is not excreted in the urine. Does this leave the body by any other channel? If not, is it metabolized in the same manner as proteid entering the organism by the normal channel?

Semmola (12) states that the injection of egg-albumen causes the appearance of proteid in the bile. The original paper was not accessible, but the reference gave no data as to the quantity excreted by this channel.

In the last metabolism experiment (XXXVII B) it was noted that the freshly passed fæces on the day following the injection were rather slimy. 5 grm. were extracted with water, and yielded coagulable proteid corresponding to 0.028 grm. nitrogen for the 16 grm. of fæces passed in 24 hours. A glance at the nitrogen of the fæces will also show that there is a tendency to an increase in the percentage of nitrogen on the first or second day following the injection.

The quantity due to the injected proteid cannot, however, be calculated, since the normal variations in the amount of fæces are so great. Thus, Rabbit A, in 2 periods of 3 days following injection excreted 2.142 grm. N in fæces; on other 6 days 1.774; an *increase* of 0.366 in the periods after injection. Rabbit B, after injection 1.467; other days 1.734, a *decrease* of 0.267 for the periods after injection.

We judge from this that the amount leaving the organism unchanged by way of the fæces must be quite small. A large amount remains to be accounted for. Stokvis (5) states that some is contained in the saliva, but this amount must be very small.

Most observations on the effect of proteid injection upon metabolism have been made upon serum. Several of the older authors mention that this is excreted completely as urea, but we could not learn how minutely the observations were made. Forster (6) made observations

on serum and egg-albumen injections; Pflüger (13) shows that in these experiments more urea was excreted than corresponded to the amount injected. Of more recent workers, Arloing (14) finds an increased excretion of N and P_2O_5, but gives no data concerning the relation of the increase to the amount injected. Albertoni (15) finds that the injection of defibrinated blood into the peritoneal cavity of starving or insufficiently nourished dogs caused no increased N excretion, if the animals had previously been well nourished, but increased the nitrogen of the urine if the animals had not been in good condition.

For egg-albumen we can only find the quoted experiments of Forster and statements of Laborde (16) that hypodermic injection into starving rabbits causes an increase of N excretion above the amount injected; but he does not give numerical data in support of his statements.

We decided to investigate this problem, and to give attention also to the form in which the nitrogen is excreted. The analyses for this purpose were made according to the methods laid down in Section II, p. 223. We shall first give a brief account of the experiments and the conclusions to which they lead, and shall follow this with the tables giving the quantitative results from which the conclusions were drawn.

We began our experiments with a dog (experiment XL). Having investigated the changes in metabolism resulting from starvation, and having demonstrated that the hypodermic injection of colloid (gum arabic) was without effect upon metabolism, we introduced subcutaneously 0.125 grm. per kilo. of egg-albumen. Neither coagulable nor non-coagulable proteid appeared in the urine, nor could any alteration in other ingredients be determined with certainty. Precisely the same negative result was obtained by the injection of respectively 0.069 and 0.125 grm. per kilo. into the ear-vein of rabbits (experiment XXIV A and B). We omit these experiments from the tables.

It appeared, therefore, that a much larger quantity needed to be injected to yield conclusive results.

We made two observations on starving rabbits (experiment XXVIII), and two (experiment XXXVII), of two injections each, on rabbits having a free supply of oats, the amount of the latter being weighed each day, and the quantity of N in the food being calculated from this.

It was found impossible to maintain a nitrogen equilibrium. In the case of the starving animals there was a rapid starvation-increase which had to be allowed for. In the fed animals, the injection produced a marked loss of appetite, so that the nitrogen balance had to be calculated from the income and output.

The results show that a considerable part of the injected proteid leaves the body unchanged. Another, smaller, portion is excreted as non-coagulable proteid. The percentage of both was much smaller in the starving animals, but this was probably because less was injected. A certain amount was excreted by way of the fæces.

Taking the rabbits of experiment XXXVII, as showing the minimum retention we have:

Of the 1.249 grm. of N (as egg-albumen) injected into the peritoneum in two doses, 5 days apart,

	Rabbit A.	B.
excreted unchanged in urine,	22.3%	32.7%
" as non-coagulable proteid in urine,	18.7%	25.5%
" as coagulable proteid in fæces, about,	5. %	5. %
Total excreted as proteid,	46. %	63. %

The remaining 54 and 37% (0.675 and 0.463 grm. of nitrogen) must have been either retained or metabolized.

It is seen from the figures that the excretion of the total nitrogen is invariably increased, and generally beyond the amount injected, so that not only is none of the proteid retained, but its injection causes the animal to lose also body-nitrogen. On this account it is not possible to state in what time this excretion of the metabolized egg-albumen occurs.

As to the form in which this is finally excreted, it is seen that the ratio of total nitrogen (excluding that of the proteids) to urea and ammonia is not changed, so that we must assume that the metabolism ends in the normal products, *i. e.* mainly urea.

TABLE VII.—METABOLISM-RESULTS OF EXPERIMENT XXVIII.

Two Rabbits were used: A ♂ 2460 grm. on July 1; B ♀ 2310 grm. They had been kept on an unlimited diet of oats and water for 10 days previous to July 2, 1900, when oats were withheld. The observations were made at 1 P. M.

	Rabbit A.						Rabbit B.					
	July 6.	July 6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.	July 6.	6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.
Day of starvation	3d.	4th.	5th.	6th.	7th.	8th.	3d.	4th.	5th.	6th.	7th.	8th.
N in faeces	....	0	0	0	0	1.105	....	0	0	0	0	0.630
N in urine, total	....	1.663	1.890	2.667	3.328	1.747	....	1.393	2.844	2.468	3.259	2.053
" urea	....	1.313	....	2.233	3.166	1.663	....	....	2.664	1.900	2.849	1.935
" non-urea	....	0.350	....	0.434	0.162	0.084	....	....	0.180	0.568	0.410	0.118
" ammonia	....	0.011	0.010	0.030	0.031	0.037	....	....	0.010	0.004	0.019	0.041
" non-coagulable proteid (bromine)	....	0.151	0.110	....	0.137	0.113	....	....	0.158	0.180	0.129	0.075
" coagulable proteid	....	....	....	0.053	0.048	0.053	....	....	....	0.126	0.133	0.050
Per cent. of total N of urine in urea	....	79	....	84	95	95	....	....	93	77 *	88	94
Ammonia	....	0.7	0.5	1.1	1.0	2.	....	....	0.4	0.1	0.6	2
Non-coag. proteid	....	9.0	6.0	....	4.0	7.	....	....	6.	7.4	4.	4
Weight = % of original weight	....	87.4	84.6	83.3	78.5	65.0	88.7	87.2	82 3	79.4	71.9	56.5
N loss = % of weight loss (exclusive of coagulable proteid)	89.8	2.77	2.70	8.89	2.77	0.5	....	4.64	2.47	3.53	1.91	0.7
				Albumen = 1.635 N injec. subcutaneously at 3.15 P. M. of 8th. 9th, Large lump at site of injection.	10th, Lump almost gone.	Found dead at 8.15 A. M. of 11th.				Albumen = 0.936 N injected into peritoneum at 3.15 P. M. of 8th.		Found dead at 8.15 A. M.

* = 81% of total N exclusive of proteid.

TABLE VIII.—METABOLISM OF RABBIT XXXVII A.

♀ 975 grm. Fed on oats. Observations made at 2 P. M.

July and August, 1900....	23 to 24.	24 to 25.	25 to 26.	26 to 27.[2]	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to August 4.
Total N consumed.....	1.047	0.261	1.396	0.863[1]	0.610	0.575	0.558	0.698	0.908[1]	0.872	0.767	0.715	8.961[1]
" excreted	....	....	1.356	1.312	1.038	0.363	0.364	0.564	0.835	0.597	0.654	0.496	7.579
Total N gained	....	....	+0.040	−0.449	−0.428	+0.212	+0.194	+0.134	+0.073	+0.275	+0.113	+0.219	+1.382
Weight gained (grm.).....	+30	−30	+40	+5.	−70	+25	−20	−10	+35	−5.	+15	+5.	+20
Difference in N = % of difference in body weight	....	....	0.1	Neg.	0.6	0.85	Neg.	Neg.	0.21	Neg.	0.8	4.0	6.9
N in faeces	0.260	0.341	0.600	0.388	0.681	0.095	0.112	0.315	0.394	0.250	0.333	0.146	....
" urine	....	....	0.756	0.924	0.357	0.268	0.252	0.249	0.441	0.347	0.321	0.350	....
N in urea.....	....	....	0.728	0.746	0.202	0.235	0.229	[0.210][2]	[0.270][2]	[0.230][2]	0.275	0.322	....
" non-urea.....	....	....	0.028	0.178	0.155	0.033	0.023	0.073	0.137	0.039	0.046	0.028	....
" ammonia	....	....	0.035	0.010	0.007	0.009	0.012	0.025	0.022	0.028	0.008	0.003	....
" non-coagulable proteid ($ZnSO_4$)	....	....	....	0.048	0.072	0.011	0.007	0.005	0.083	0.045	0.025	0.037	....
" rest (theoretically alloxur, etc.)	....	....	....	0.049	0.006	0.006	0	....	Neg.	....	Neg.	Neg.	....
" coagulable proteid.....	....	....	....	0.071	0.070	0.007	0.004	....	0.071	0.038	0.018	....	0.279
% of total nitrogen of urine (exclusive of proteids) in Urea.....	....	....	96	89	79	90	92	....	71.[3]	78.[3]	88.	92.	....
Ammonia.....	....	....	4.6	1.2	2.5	3.4	4.8	10.	6.0	9.	2.7	0.9	....
Non-coagulable proteid.....	....	....	....	5.5	25	4.2	2.8	2.	22.	12.	8.	11.	....
				At 2.45 of 26th albumen = 0.680 N into peritoneum.					At 2.37 of 31st albumen = 0.559 N into peritoneum.				

[1] Including injected. [2] Some urine lost, not over 10%; no correction applied. [3] Direct analysis missed; estimated by difference.

TABLE IX.—METABOLISM OF RABBIT XXXVII B. ♀ 1185 GM.

Observations made at 2 P. M.

July and August, 1900....	23 to 24.	24 to 25.	25 to 26.	26 to 27.	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to Aug. 4.
Total N consumed.....	1.134	0.872	0.959	0.951 [1]	0.523	0.872	1.221	0.959	0.646 [1]	0.785	0.802	0.593	10.315 [1]
" excreted	1.352	0.894	1.213	1.494	0.981	0.559	0.631	0.715	0.750	1.093	0.693	0.743	11.118
Total N gained	−0.218	−0.022	−0.254	−1.233	−0.458	+0.313	+0.590	+0.244	+0.663	−0.310	+0.109	−0.150	−0.803
Weight " (grm.)	+10	−10	0	0	−40	+25	+40	−15	+5	−30	+5	−15	−25
Difference in N = % of difference in body-weight	Neg.	0.22	....	.....	1.1	1.2	1.5	Neg.	Neg.	1.	2.	1.	3.2
N in faeces	0.468	0.290	0.230	0.358	0.178	0.259	0.201	0.306	0.292	0.207	0.173	0.239	
" urine	0.884	0.604	0.983	1.136	0.803	0.300	0.430	0.409	0.458	0.886	0.520	0.504	
N in urea.....	0.805	....	0.917	0.941	0.566	0.260	0.396	0.390	[0.338 [3]]	0.773	0.454	0.469	
" non-urea	0.079	....	0.066	0.195	0.237	0.040	0.034	0.019	.....	0.113	0.066	0.035	
" ammonia	0.011	0.006	0.005	0.008	0.012	0.008	0.005	0.014	0.010	0.009	0.011	0.003	
" non-coagulable proteid ($ZnSO_4$)	....	....	....	0.067	0.125	0.011	0.010	0.011	0.053	0.097	0.022		
" rest (theoretically alloxur, etc.)	....	....	....	0	0.004	0.005	0.010	Neg.	.....	Neg.	Neg.		
" coagulable proteid ..	....	....	....	0.120	0.096	0.006	0.009	.....	0.056	0.072	0.050	.....	0.409
% of total nitrogen of urine (exclusive of proteids) in													
Urea	91	....	93	84	80	87	94	97	84	95	97	93	
Ammonia	1.2	1	0.5	0.8	1.7	2.6	1.2	3.5	2.5	1.1	2.3	0.6	
Non-coagulable proteids..	....	....	....	6.0	17.6	3.6	2.4	2.7	13.2	11.9	4.7		
				At 2.50 of 26th injected Albumen = 0.690 N into peritonem.					At 2.40 of 31st injected Albumen = 0.559 N into peritonem.				

[1] Including injected.

[3] Direct analysis missed; estimated by difference.

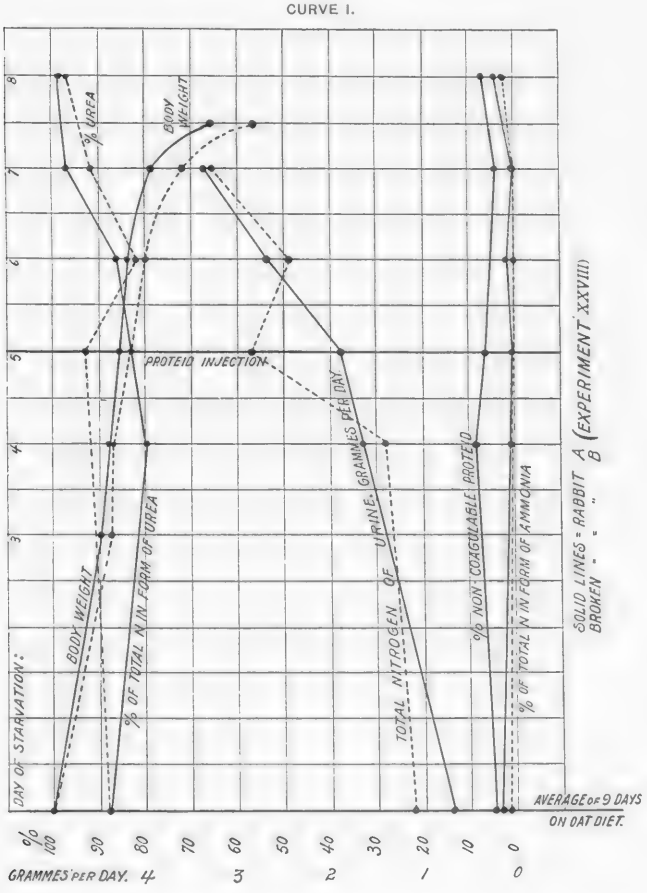
The presence of *non-coagulable proteid* as the result of hypodermic and intraperitoneal injections is an interesting fact. It was established by the ferrocyanide reaction after boiling, as also by the bromine and zinc-sulphate methods, so that it seems beyond doubt that these injections may cause the excretion of a substance having these properties. The amount was not sufficiently large to permit further characterization.

No such substance appeared in the case of any of the intravenous injections in which it was sought; nor did it occur in all cases of hypodermic injection. Its excretion sometimes persisted for a very long time—especially after repeated injections. In experiments XXXVII A and B, in which it was estimated quantitatively, the amount was proportional to the amount of coagulable proteid excreted (A: coagulable = 22.3% of the injected, non-coagulable = 18.7%. B: coagulable = 32.7%, non-coagulable 25.5%). There was no suppuration in these cases, so that this theory of its origin may be discarded. The fact that it did not occur on intravenous injection also disposes of the theory that it might result from the fever.

We believe that these albumoses represent a transition stage in the metabolism of the injected proteids. This would be in line with the observation of Krehl and Matthes (17) that deutero-albumoses are formed throughout the fever period, when there is also an increased proteid metabolism. The fact that it is not seen after intravenous injection can perhaps be explained by so much proteid being excreted unchanged that the organism may metabolize the small residue completely. This agrees with the fact that none is excreted after very small hypodermic injections. The fact of its long persistence in some cases favors the view that albumen injection may cause a long persisting alteration in proteid metabolism.

Experiment XXVIII gives good illustrations of the effect of *starvation on rabbits.* This subject has been investigated by Rubner (18), and more recently and thoroughly by Heymans (19), who draws his observations from 32 full grown rabbits, after withdrawal of both food and water. Our observations, which we present in the form of Curve I, show agreement with the latter observer as regards loss of weight (35 and 43.5%), the curve of loss of weight declining suddenly on the last day; and in the premortal rise of nitrogen

excretion. We wish to call particular attention to the slight change in the nitrogen during the first four days.


CURVE I.
% UREA
BODY WEIGHT
PROTEID INJECTION
BODY WEIGHT
% OF TOTAL N IN FORM OF UREA
TOTAL NITROGEN OF URINE. GRAMMES PER DAY
% NON-COAGULABLE PROTEID
% OF TOTAL N IN FORM OF AMMONIA
SOLID LINES = RABBIT A
BROKEN " " " B (EXPERIMENT XXVIII)
DAY OF STARVATION:
AVERAGE OF 9 DAYS ON OAT DIET.
GRAMMES PER DAY. 4 3 2 1 0

This agreement is remarkable in view of the fact that our animals were supplied freely with water. On the other hand, death occurred between the 7th and 8th day, whereas in Heymans' experiment it happened on the 6th to the 26th, averaging 15 to 16 days. That the early fatality might be connected with the proteid injection seems very improbable, since the loss of weight is so nearly the same as in Heymans'

observations. Some of Rubner's animals (18) also succumbed as early as the 4th day. Other instances are quoted by Kaufmann (21). We are inclined to refer it rather to the fact that our animals were young, and that their metabolism therefore was more active. The early occurrence of the starvation-rise in the nitrogen excretion, points in the same direction.

A striking feature in our cases is the fact that the proportion of urea to total nitrogen is notably increased in the last days of starvation.

Heymans also noted that it was higher during starvation than before, but since he made the urea determinations by a rather rough hypobromite test, he did not feel himself justified in laying stress upon the small difference. We believe that our methods are sufficiently trustworthy, and although the number of experiments is very small, yet they seem to justify the conclusion that the nitrogen metabolism in starvation tends to more complete oxidation than normally. This tends to support Voit's explanation of the cause of the premortal rise in nitrogen excretion, *i. e.*, that it is due to the body becoming relatively poor in fat; as against F. N. Schulz (24), who accounts for it by more proteid being thrown into the circulation by the extensive dying of cells; or another theory, which supposes that the cells become incapable of utilizing fat. With either of the last two theories, one would rather expect the oxidation of the proteids to be less complete.

V. PHYSIOLOGICAL AND OTHER PHENOMENA.

These will be considered in the following order:

A. Effects in Non-Fatal Cases.

1. Respiration and Circulation.
2. Temperature.
3. Diuresis.
4. Glycosuria and Hæmoglobinuria.
5. Histological Findings after Injection of Egg-Albumen.

B. Fatalities Occurring in the Experiments and Their Causes.

Anæsthetics.

Bacterial Infection.

Toxic Effects of Egg-Albumen.

Toxic Effects of Alkali-Albumin.

Myosin.

A. EFFECTS IN NON-FATAL CASES.

1. *Respiration and Circulation.*—During the earlier experiments on intravenous injections it was always noticed, incidentally, that the respiration increased. In two experiments (XXII and XXVI) special attention was given to this phenomena; tracings were also taken from the carotid artery and the venous pressure was measured by a water manometer connected with the central end of the femoral vein. In one animal the injection of egg-solution was preceded by an equal amount of normal saline, to allow of a comparison of the two. It was found that the effects were essentially the same in both cases. The respiration was deepened, the heart slowed and strengthened, both the arterial and venous pressures showing a small rise persisting through some 15 minutes. It may, therefore, be stated that intravenous injection of egg-albumen has no effects upon respiration and circulation beyond those of the liquid with which it is introduced.

This result differs considerably from that obtained with sera of foreign species (Arloing (14), Weiss (11), Friedenthal and Lewandowsky (10), &c.). Thus Arloing (14) describes after injection of horse serum into dogs a stimulation of the respiratory centre and a depression of the cardiac muscle and vascular tone. T. G. Brodie (25) finds that the intravenous injection of serum of any source into cats causes arrest of respiration, inhibition of the heart, and vasodilation. All these phenomena last for some time and are produced reflexly by stimulation of the pulmonary branch of the vagus. The active substance is an albumin, coagulating at 86° C., produced during the clotting of the blood. Repetition of the injections leads to immunity. These results are obtained only on the cat. Nothing of the kind was ever seen by us with egg-albumen or its modifications or with muscle on dogs or rabbits.

It must be remembered that the proteid content of our solution was only $\frac{1}{4}$ to $\frac{1}{2}$ as strong as that of serum. Stronger solutions are impracticable, since a proteid content of 10 to 15% causes speedy death from embolism, preceded by dyspnœa, then respiratory paralysis and lastly cardiac stand-still. Two deaths from this were recorded (experiments XVII and XLI). Another death (experiment XIV) from what was probably embolism from injected air occurred in four hours after injection, with symptoms of progressive medullary paralysis.

2. *Temperature.*—The statement is found in text-books (Lazarus-Barlow, General Pathology) that injections of proteids—particularly

albumoses, but also others—cause a rise of temperature. Quincke (26) notes this for the intravenous injection of defibrinated blood. Injection of other substances may also cause hyperpyrexia. Thus E. Haack (27) noted it after the subcutaneous injection of silver nitrate and tincture of iodine; these substances, however, cause simultaneously an albumosuria. Combemale and Mouton (28) found that the hypodermic injection of 20 cc. of normal salt solution produced a rise of temperature fairly regularly in tuberculous patients, but not in healthy individuals, nor in those suffering from other diseases. For animals, however, Thompson (29) has shown that the intravenous injection of small quantities of normal saline caused a rise of temperature to 2° C. Bose and Vedel (30) have seen the same effects from larger injections.

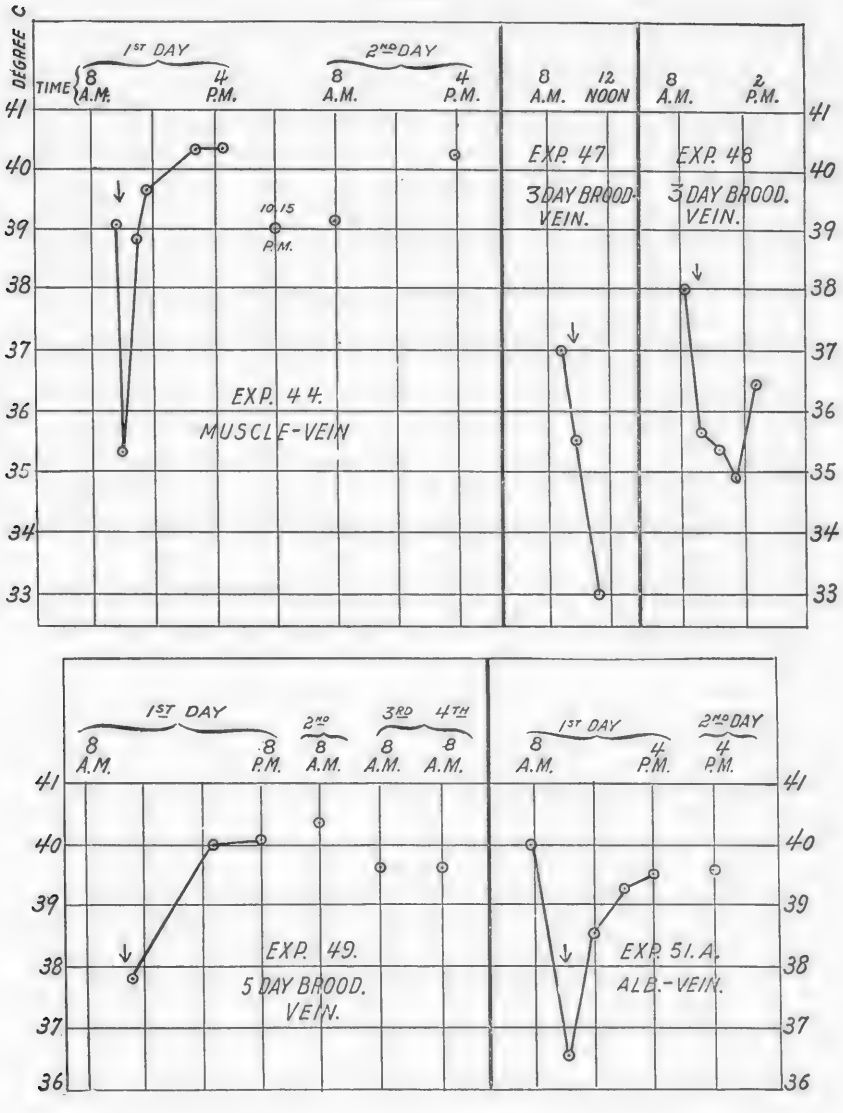
In a number of our experiments on rabbits we directed particular attention to the effects of various injections upon the temperature. A self-registering thermometer was inserted for a depth of 7 cm. into the rectum, and left in position until no further rise occurred. The results are represented in Curves II on Plates XVI-XIX. The arrow indicates the time of injection. Urethane was given in all the cases of intravenous injection

It will be seen that all the proteids which were tried (albumen, derived albumen and myosin) produce a very distinct rise of temperature. This occurs whether they are injected subcutaneously, or into the peritoneum, the ear-vein, or the jugular vein. In the last, the rise is often masked by the fall of temperature due to the anæsthetic.

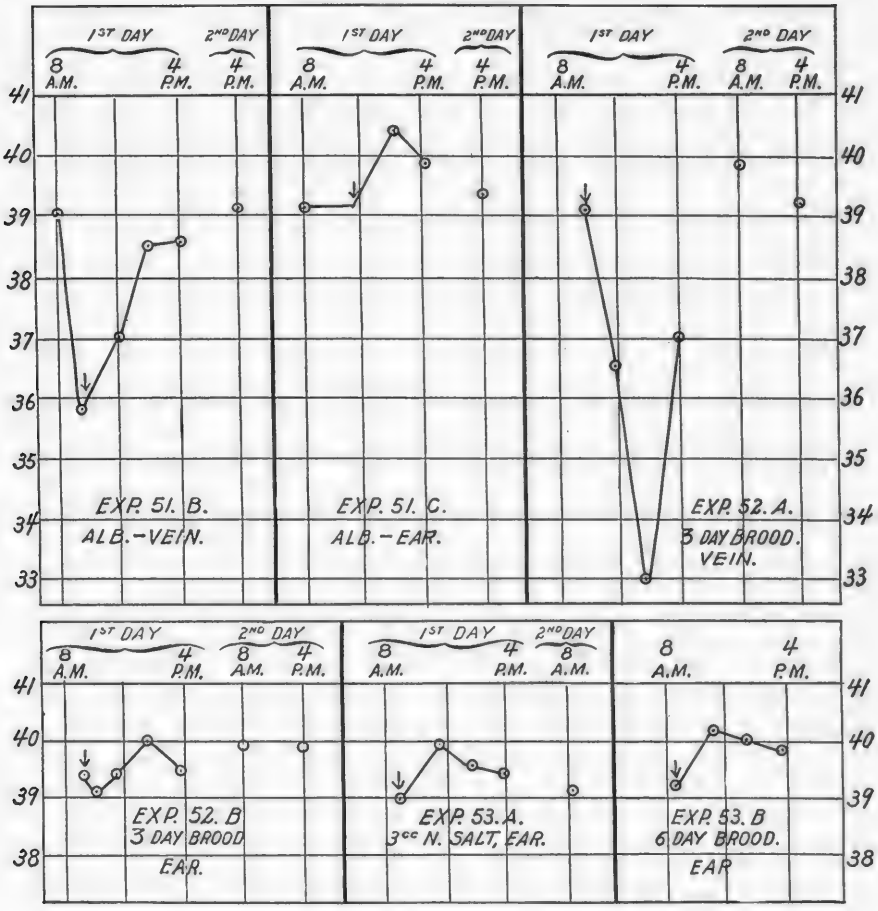
When the amount of the latter was very large, and especially when it was fatal, it prevented the rise entirely. Urethane alone, 0.5 grm. per kilo. by rectum, lowered the temperature 2.3° C. The existence of the bacterial infection, presently to be described, had no influence on the fever, either as to its height or duration. All the proteids had about the same effect.

The rise begins in about an hour after the injection of the solution, and reaches its maximum in from 6 to 24 hours (usually 6 to 8 hours). It then falls very rapidly, but in rare cases may persist for 2 or 3 days (Experiment 62, Plate XIX). A recurrence of the fever is

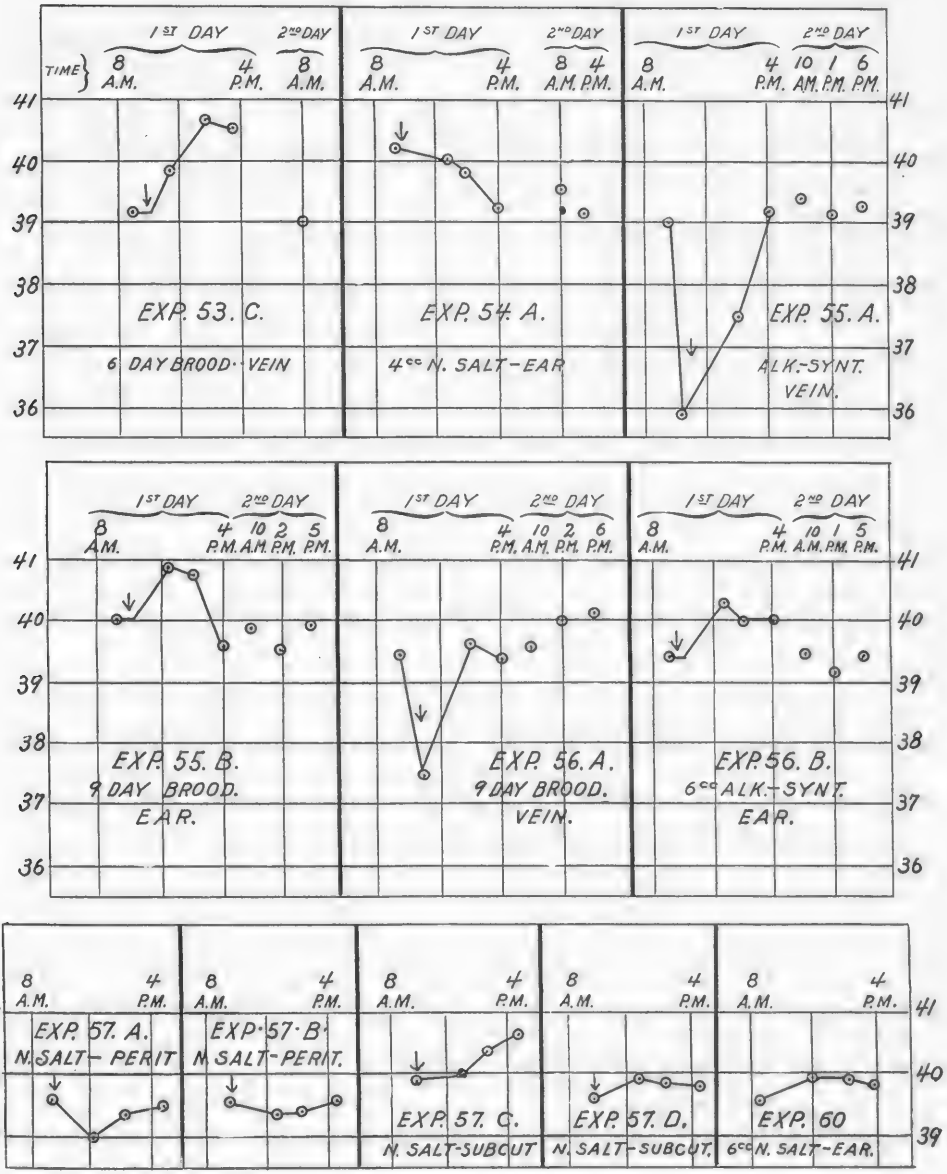
CURVES II.


DEGREE C
TIME
1ST DAY
2ND DAY
8 A.M.
4 P.M.
8 A.M.
4 P.M.
10.15 P.M.
EXP. 44.
MUSCLE-VEIN
8 A.M.
12 NOON
EXP. 47
3 DAY BROOD.
VEIN.
8 A.M.
2 P.M.
EXP. 48
3 DAY BROOD.
VEIN.
41
40
39
38
37
36
35
34
33
1ST DAY
2ND
3RD
4TH
8 A.M.
8 P.M.
8 A.M.
8 A.M.
8 A.M.
EXP. 49.
5 DAY BROOD.
VEIN.
1ST DAY
2ND DAY
8 A.M.
4 P.M.
4 P.M.
EXP. 51. A.
ALB.-VEIN.
41
40
39
38
37
36

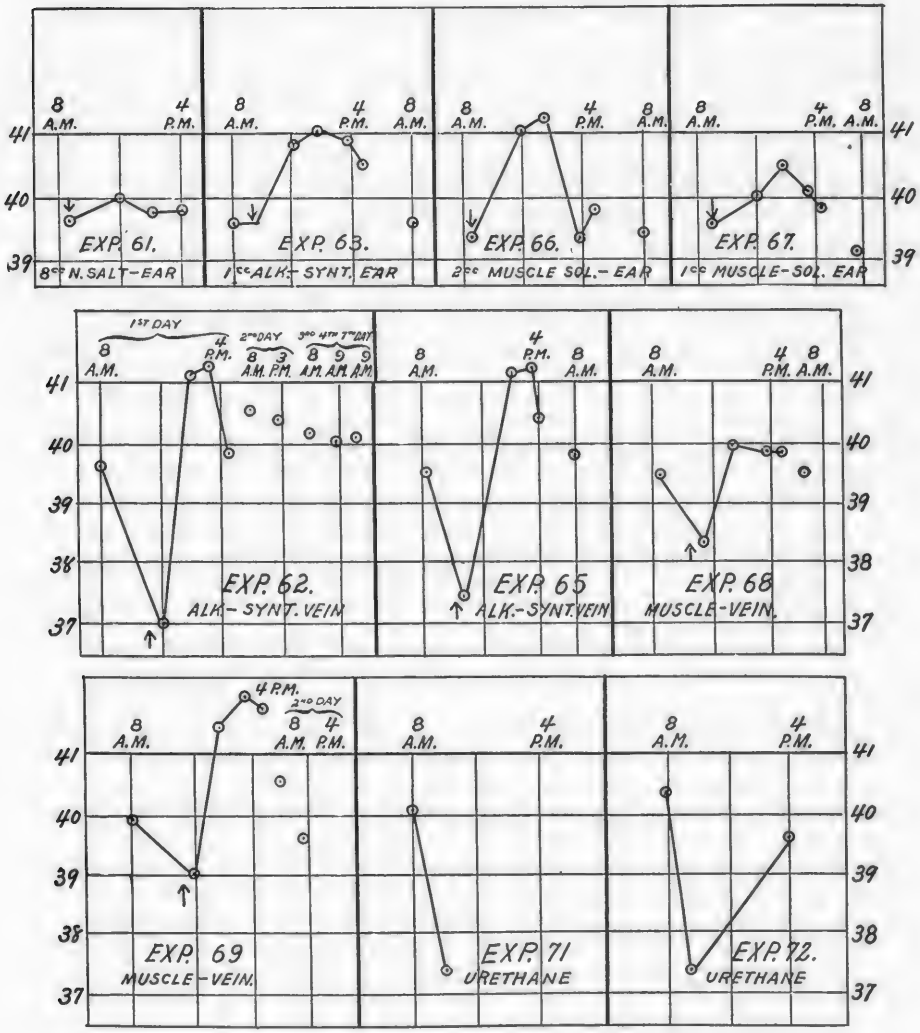
CURVES II—Continued.



CURVES II—Continued.



CURVES II—Continued.



sometimes seen on subsequent days, particularly in the afternoon. The rise is usually a trifle over 1°, but may be as much as 2° C. It seems to be almost independent of the quantity injected; at least, it may be produced by extremely small quantities (as in experiment 66 by 15 mg. of the proteid of rabbit's muscle, per kilo, of injected rabbit).

Normal salt solution alone, when injected into the ear, may cause some rise of temperature, but smaller than that following the use of the proteïds (the mean being 0.35° C.). Subcutaneous injection may also cause a rise, but this is still smaller. In some cases the injection of normal salt solution, by way of the ear, skin or peritoneum, causes a slight fall of temperature (from reflex dilatation of the vessels?).

3. *Diuresis*—The injection of egg-albumen tends to cause a diuresis. In certain animals an anuria persists for many hours, but this may be seen without albumen injection, and even after the injection of a third of the body-weight of normal saline. The curve of the diuresis, as recorded in two cases, differs very greatly from that produced by the injection of normal salt solution (Sollmann 31).

With the latter the flow of urine reaches its maximum in ½ hour and has returned to a relatively small figure in 1½ hours. With albumen injection, on the other hand, there is a small primary rise, reaching its maximum in 15 to 20 minutes, and then a fall to 50 minutes. This curve is probably attributable to the salt solution which carries the proteid, since it is practically identical with that produced by salt solution alone.

About 50 minutes after the injection, however, a second diuresis sets in, reaching its maximum in about 2 hours, when it is 3 to 5 times greater than the primary rise. This must be due to a specific irritation of the kidneys by the albumen. We did not investigate whether this action is exerted upon the vessels or cells. The accompanying Curve III, from experiment I, gives a typical illustration of this phenomenon (Plate XX).

4. *Glycosuria.*—This never occurred on hypodermic or intraperitoneal injection. It was noted in the case of dogs, after intravenous injection, in 5 out of 18 animals. The quantity was deter-

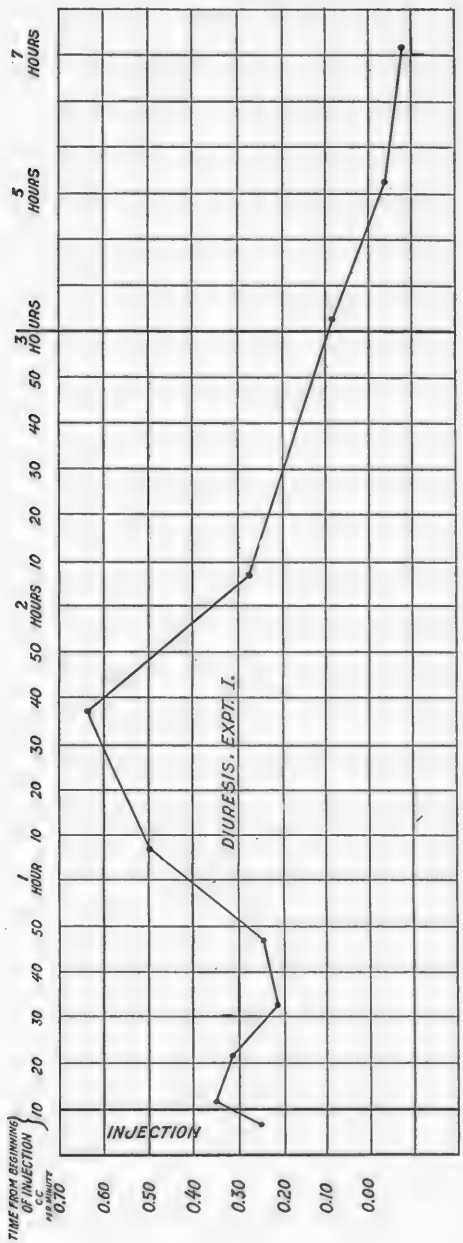
mined in but two experiments (I and III). In the latter it reached 0.32 grm. per kilo. The excretion was completed inside of the first 24 hours, except in one experiment (XXI) in which it appeared on the second day after injection, the urine being free from it on the first. In this animal, morphine alone caused an abundant glycosuria.

Evidently the excretion of sugar must be referred to the anæsthetic and to the morphine. Injection of the proteids does not cause glycosuria.

Hæmoglobinuria.—This was observed in only one case (experiment XII) and could not be attributed to the injection of albumen. The urine remained normal for 7 days after a second hypodermic injection, when it suddenly showed oxyhæmoglobin and methæmoglobin, a strong content of albumin, but no casts. On the following day these had disappeared, but the urine now contained bile pigment. The animal was sacrificed on this day. The autopsy was negative, and microscopic examination of the kidneys did not reveal anything abnormal.

In one experiment on a rabbit (No. 48) the bladder at autopsy was found distended with a reddish urine, giving the oxyhæmoglobin spectrum. Microscopic examination showed the abundant sediment to consist of numerous red blood-cells and a large quantity of granular casts. These appearances were not present in any other experiment of the entire series. The animal, which had received some albumen-solution intravenously, had died 3½ hours after the injection, evidently from the effects of an overdose of the anæsthetic (urethane, 1.18 grm. per kilo.; temperature fell to 35° C.). The peritoneum contained a large quantity of free blood, which clotted on removal. A rupture of the liver was noted, but it could not be decided whether this was inflicted at the autopsy (the liver tissue being extremely friable); or whether it had occurred earlier, perhaps during the injection. The microscopic examination showed considerable degeneration of the renal and hepatic epithelium. The last was certainly due to the urethane. The urine, which was of neutral reaction, showed a large quantity of coagulable proteid, but no sugar. This fact shows that the albumen was not due to the hæmorrhage, but that the excretion of the injected albumen was well advanced at the time of death. The marked nephritis, shown by

CURVE III.


7 HOURS
5 HOURS
3 HOURS
2 HOURS
1 HOUR
10
20
30
40
50
10
20
30
40
50
10
20
30
40
50
10
20
30
40
50
60
70
INJECTION
DIURESIS. EXPT. I.
TIME FROM BEGINNING OF INJECTION
C.C. PER MINUTE
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00

the casts and by the histological appearances, can not have been due to the injection. Nor do we think that it could have been caused by the urethane, and we must leave it unexplained.

5. *Histological Findings After Injection of Egg-Albumen.*

Previous experimenters have found that the injection of proteids causes slight, but distinct, nephritic changes. Semmola (12), Laborde (16) and Seigert report parenchymatous nephritis after injection of egg-albumen. Laborde also mentions necrotic foci in the liver; Semmola reports retinal changes. O. Weiss (11), injecting foreign serum into dogs, finds only hyperæmia of the renal cortex. A. Pettit (32) remarks that the injection of eel serum causes in rabbits a rapidly progressing degeneration of renal epithelium, noticeable even when the animal survives only a few minutes.

We were able through the kind assistance of Professor Wm. T. Howard, Jr., to subject the greater part of our material to histological examination. The methods employed have been described in Section II (p. 218). The animals were killed at various times, but usually several days after the injection.

The *kidneys* of nearly all the animals examined showed a greater or less degree of congestion of both cortex and medulla. Those of the dogs, after injections of plain albumen, presented no noticeable degeneration. In the rabbits on the other hand, the epithelium of the convoluted tubules was almost always cloudy and swollen, sometimes even hyaline or vacuolated. Granules of iron-free pigment were seen in the epithelial cells in many cases. Hyaline casts, resulting from coagulation of the albuminous urine, were often seen in the tubules. These changes, except the last, are such as we often saw in animals which had received no injections. It is quite possible, though by no menas certain, that they are more intense after the injection of the proteids.

In experiment 48 alone, described on page 244, was the degeneration at all extensive. This was also the only case in which casts were at all numerous in the urine. The cause of the peculiarities of this experiment must stand unexplained.

We may say, then, that the injection of egg-proteids does not lead to any conspicuous nephritic lesions. This holds true for dogs, rabbits and guinea-pigs. A slight degree of cellular degeneration cannot be excluded. The constant appearance of congestion points to some inflammatory action. Certain experiments give further evidence

of nephritic changes through a long continued albuminuria, through an acute globulinuria, etc.; but these changes are evidently not of such a degree as to give rise to marked degenerative lesions.

The intravenous injection of an extract of rabbit's *muscle* into two rabbits (experiments 68 and 69), showed very marked and extensive degeneration of the renal epithelium. The amount of urethane was very small in these cases (0.33 to 0.35 grm. per kilo.), so that the changes could not be attributed to it. Moreover, the other organs were normal. The degeneration was much more marked in the animal killed three days after injection, than in the one killed after 10 days. The kidneys showed no alterations in a case (experiment XLIV) of intravenous injection of dog's muscle and in experiment XXXIII of intravenous injection of human muscle into rabbits; nor in 2 rabbits (experiment XXXVIII), into whose peritoneum chicken's muscle was injected; nor in two others (experiment XXXIX), which received dog's muscle in the same manner. The degeneration of the renal epithelium, as noted in experiments 68 and 69, was therefore peculiar to this particular extract, and is not a common sequence of injections of muscle extracts.

The absence of conspicuous pathological lesions holds true still more for *other organs*. The spleen, intestines, pancreas and adrenals showed at most some congestion. The liver epithelium was very often greatly degenerated in rabbits which had received large doses of urethane; but this was plainly referable to the latter. Parasitic changes were often seen, and coccidium oviforme was very often found in the bile-ducts. In experiment 65 numbers of amœba-like parasites were seen in one kidney.

The absence of conspicuous degenerations is of interest not merely from its connection with the effects of proteid injection, but also because it bears on the much discussed question, whether uncomplicated hyperpyrexia causes cellular degeneration. We have not seen any such after elevations of temperature of one or two degrees lasting for two days. This tends to support Naunyn's view, corroborated lately by E. v. Czyhlarz (33), who saw no degenerations after cerebral puncture in ten rabbits.

Some attention was given to the distribution of pigment. Both hæmofuscin (iron free) and hæmosiderin (iron-containing) were found, in variable amount, in all cases. The latter was exclusively in the spleen. The former in the liver epithelium, in the spleen, both free and in cells, and often in the renal epithelium.

B. FATALITIES OCCURRING IN THE COURSE OF THE EXPERIMENTS AND THEIR CAUSES.

Many of our animals succumbed in the course of the experiments. The greater number of these deaths could be referrd to well defined causes. Amongst these are embolism through two great concentration of the solution or from air; overdoses of the anæsthetic; bacterial infection; excessive handling or long exposure to unhygienic conditions, etc. A certain number pointed to a specific toxicity of the proteid. It seems to us of interest to give an illustrative description of the course and of the gross and microscopic changes in the principal fatal cases.

The phenomena of embolism are dealt with on page 241.

Deaths from the Anæsthetic.—Only one late death was observed in a dog (experiment XIX) which had received morphine and ether.

Experiment XIX.—This animal was very listless on the day following the operation; later it lay almost motionless in a comatose condition, and was found dead on the following morning. Autopsy negative.

The majority of deaths occurred in rabbits, after chloretone or urethane. The fatal dose for *chloretone* by stomach catheter, lies slightly above 16 cc. of a saturated solution per kilo. The symptoms were very similar to those produced by urethane. The degeneration of the liver cells was absent, nor were there any other histological lesions. There was, however, in all cases a marked capillary congestion of the abdominal organs.

The fatal dose of *urethane* given in 5% solution per rectum, lies between 0.75 to 1.0 grm. per kilo. But doses of 0.6 grm. cause very severe degeneration of the hepatic cells. Franz Müller (37), found that 1 grm. of urethane per kg. killed one rabbit out of three (in 2 days). When given by mouth the fatal dose of these anæthetics lies somewhat higher than by the rectum.

The following experiment forms a typical illustration:

Experiment XXXIII.—July 18, 1900. Rabbit ♂, 1790 grm., was anæsthetized with 1.5 grm. urethane, and ether; the jugular vein was exposed under aseptic precautions, and 125 cc. of a solution of human muscle in normal salt solution (= 0.486 grm. proteid) were injected.

The animal was partly conscious at the end of the operation (2.30 P. M.) and appeared in good condition. The wound was sutured and the rabbit placed in its cage. At 5.30 it was found lying on its side, very much worse. There had been, and was at this time, a profuse diarrhœa and

involuntary passages of a small quantity of urine. The heart was very fast; respiration 140; temperature 36.1° (normal rabbit = 40° C.); pupils of medium size, ear-vessels normal; reflexes slow. At 7 p. m. the respiration was 140; temperature 35.1°, heart fast, reflexes lost except corneal; pupil rather small. Heated bricks were placed about the animal at 7.15. At 7.25 the respiration became slowed to 12 and was coughing and spasmodic; temperature 34.4°. The animal died at 7.28, the respiration ceasing before the heart.

The *autopsy*, made immediately after death, showed a congestion of all abdominal organs. The small intestine was contracted and contained mucous fluid. The bladder was empty. The lungs were pink; the heart was not distended. The liver and kidneys presented a peculiar waxy appearance.

Histological Examination: (This refers to all the experiments in which urethane was used in a dose of 0.6 grm. per kg. or larger. With a dose of 0.35 grm. per kg. there were no noticeable lesions).

The principal changes are found in the *liver*. There is an intense diffuse degeneration of the hepatic epithelium, more marked in foci. The cells stain unevenly, are very granular, and present numerous vacuoles. Fatty degeneration is also sometimes seen. The nuclei are fairly normal. The perilobular tissue is normal. After large doses a congestion of the central vein and capillaries is also seen. The process of degeneration must be a very rapid one, for it is fully developed in animals which died 1½ hours after the injection (experiment XLVII). In the kidneys, some degree of congestion is common. The epithelium is always somewhat granular, but not more so than may be encountered in normal animals. The spleen is always congested. The other organs—intestine, medulla oblongata, pancreas, cardiac muscle, adrenals, are normal. The distribution of pigments shows nothing unusual.

Deaths from Bacterial Infection.—During May, 1901, an epizootic made its appearance amongst the guinea-pigs and rabbits at the laboratory, and carried off a great number of the former. The mortality was not so great with the rabbits, perhaps because they were not kept sufficiently long. With these animals, the disease was not suspected until the sections were examined. The disease occurred in animals which had not been handled, and was therefore not due to the injection.

The *microorganisms* as seen in the tissues were for the most part rather short pleomorphic bacilli, but larger numbers of cocci were often seen, especially in the lungs. Agar cultures were made from the guinea-pigs, but could not be examined for several weeks. They then showed a number of organisms, of which only B. coli communis could be identified. We are indebted to Mr. H. J. Gertenberger for these cultures.

The rabbits, even when intensely infected, showed but slight symptoms

during life. The guinea-pigs became listless on the last day, did not eat, lay on their side, and had a gasping respiration. The *autopsy* itself did not show any conspicuous changes. The numerous cases which were examined permit us to pursue the histological lesions through the successive stages of the infection. The bacteria were seen in all the tissues. The kidney showed some change in the epithelium of the convoluted tubules; the cells becoming somewhat swollen and cloudy or hyaline. There were no casts. The connective tissue was often fairly abundant. With more intense infection, the cell degeneration became very conspicuous. The connective tissue showed considerable round-celled infiltration. Gas-spaces were discernible. The liver cells showed at first a moderate, diffuse, granulation. In the perilobular tissue there was frequently more or less pronounced infiltration with young connective-tissue cells. The degeneration of the epithelium became very profound with the progress of the infection, being also somewhat focal. There were severe congestions and hæmorrhagic areas, the latter containing hæmosiderin. The spleen was congested, but otherwise normal even in the severe grades. The cardiac muscle was very profoundly affected, containing a mass of bacteria, and its structure having largely disappeared. The lungs were congested; the alveoli, bronchi, and blood contained many bacteria. The intestine, adrenals and pancreas were normal.

There was some hyperleucocytosis, and an increase of eosinophiles, especially in the spleen. Hæmosiderin and hæmofuscin were unusually abundant in the more marked grades of infection, especially the hæmosiderin in the spleen.

Toxic Effects of Egg-Albumen.—The following very striking instance of death following the intravenous injection of egg-albumen, which occurred rather early in the course of the research, directed our attention to the possibility of a toxic action of proteids which are usually considered harmless, and led us to an extensive experimental investigation of this subject.

Experiment XVIII.—This dog was but lightly anæsthetized during the operation. On the day following it was lively, ran about, and seemed perfectly well. It remained so during three days, and even at 8 A. M. on the 4th day nothing abnormal was noticed. The animal was not observed again until 1 P. M. of the 4th day, when she was found in extreme clonic spasms and almost insensible. Restorative measures were resorted to, but failed, death occurring at 1.30 P. M. The autopsy and histological study were both negative. The wound had suppurated a little.

This case is particularly interesting, being the only one in which an animal died rather suddenly after several intervening days of perfect good health. It seemed to us worth while to attempt to reproduce the condition, to enable us to study it more carefully.

As we had seen this course but once in more than 20 animals which we had injected, we searched for methods of increasing any existing toxicity of the egg-albumen. For this purpose we first tried the effect of repeating the injections on animals which are usually very sensitive to toxic agents.

We chose 6 guinea-pigs for the purpose, injecting them always in pairs, to exclude other accidental causes so far as possible. The injections were sometimes made under the skin, sometimes into the peritoneum. We were always successful in obtaining a fatal result, both animals of a pair always dying at the same time. Death usually occurred during the night succeeding the last injection, so that the final symptoms could not be observed. The guinea-pigs were always somewhat depressed after each injection, but nothing else could be noted. The autopsy showed nothing abnormal. There was no sign of peritonitis. However, the number of injections required, as well as the amount of proteid, were so large, that it seemed unjustifiable to refer the death to them. We were forced to conclude that the injection of fresh egg-albumen was in no way toxic.

Mariani (1897) has shown that starving rabbits treated with hypodermic injections of egg-albumen die before the control animals, but this might be referred to the increased nitrogen metabolism produced by such injections.

We may state at this place that the injections ordinarily produced few or no symptoms in either dogs or rabbits, except that the animals tended to become emaciated while preserving a ravenous appetite.

The phenomena of experiment XVIII must therefore have been due to the presence of some extraneous toxin in the egg-albumen. It remained to determine whether this was due to infection of the egg-solution, or whether such a toxin could be developed in the unspoiled egg itself. The eggs used for this experiment were purchased in July, in the open market, and were of unknown age. Whilst the eggs were not visibly spoiled, the conditions were distinctly favorable to fermentation. The cholin, etc., of the yolk would form a favorable starting point for such ptomains. To decide this question, we took some freshly laid eggs and placed them under conditions which would be most conducive to such change, *i. e.* in a thermostat

at 40° C. Here they were kept for 3, 5, 6 and 9 days. In the last case, the yolk could not be separated, and had to be included in the solution. Animals injected with solutions made from these brooded eggs behave precisely as if injected with normal egg-albumen. Only one case ended fatally, and this five days after the injection. The histological examination showed thrombosis of veins in the liver, perhaps quite sufficient to cause death; but we doubt whether this result could have been due to the injection.

We conclude that pure egg-albumen is not at all toxic, nor does it develop a toxicity by prolonged sojourn at brooding temperature.

This leaves bacterial infection as the explanation of experiment XVIII. Bacteriological examination was unfortunately neglected, but the tissues were certainly not conspicuously invaded by any micro-organism. The symptoms resembled superficially those of traumatic tetanus; since the wound was open, infection by this channel could have occurred quite easily. However, the sudden outbreak, the general distribution of the convulsions and rapid fatality speak very strongly against the existence of bacterial tetanus. The absence of any such effects in other animals operated upon, and kept under precisely the same conditions, makes it unlikely that the infection occurred by way of the wound. The toxic agent must have been introduced with the injected solution.

(Fiquet (34), 1899, claims that even albumoses and peptones are not toxic on intravenous injection, when prepared sufficiently pure, and that they then have no influence on the clotting of blood. He refers the ordinary action to admixture of toxalbumins and ptomains).

Toxic Effects of Alkali-Albumin.—Only one fatal case occurred in six intravenous injections of alkaline egg-syntonin in dogs, and two in rabbits. In this fatal case the alkalinity of the solution was unusually high, and the amount large (35 cc. per kilo. of a solution containing 0.376% of free NaOH). The same symptoms and lesions could be elicited in another animal by the injection of pure $\frac{N}{10}$ NaOH solution, so that they are not dependent upon the proteid. We reserve their detailed description for another paper.

Briefly, the animals died in about 6½ hours, in coma and with convulsions. The autopsy revealed intense congestion of the abdominal

organs, particularly of the large intestine, with bloody effusions into the lumen of the alimentary canal and into the peritoneum.

Histologically, the main lesions were a necrosis of the intestinal villi and an acute interstitial nephritis, in every way similar to that described for the human subject by Councilman (35) and Howard (36). Hæmorrhagic areas were found in various situations. There was a profound hæmochromatosis, consisting mainly in the deposition of iron-free pigment granules in the intestinal mucosa, the liver, the blood, the lymph channels, and in the renal epithelium.

Other animals, which had received less alkali, showed much less marked changes in the same direction, or were free from lesions. Alkali-albumin, therefore, does not differ in its histological results from native albumen.

Muscle Extracts.—These did not possess any specific toxicity; the histological alterations corresponded to those seen after injection of egg-albumen.

VI. CONCLUSIONS.

The conclusions which we derive from our observations are as follows:

1. The excretion of injected egg-albumen as such is in no case complete. The quantity retained varies from 23 to 100%.

2. The amount retained varies:

   a) directly with the slowness of absorption. This is determined by the manner of administration.

   b) directly with the time during which the proteid remains in the body; and therefore inversely to the rapidity of excretion.

   c) inversely to the quantity injected; this has however much less effect than (a) or (b).

   d) with individual peculiarities; but these are not very conspicuous.

3. The excreted proteid coagulates at the same temperatures as the injected albumen.

4. Injection of egg-albumen does not cause the appearance of globulins in the urine.

5. The proportion of proteid coagulating at lower temperatures is less in the urine than in the injected solution. When a solution has been heated to 73° before injection, the urine also does not coagulate below this temperature.

6. Egg-albumen injected into the hen is excreted as with mammals.

7. The albuminuria lasts in typical cases from 1½ to 3 days, according to the manner of administration.

The excretion begins very shortly (7 minutes) after injection. 37 per cent of the total proteid injected may be excreted in an hour. About three-fourths of the total excretion takes place within the first 17 hours; the excretion is almost completed in the next 15 hours, only traces being excreted thereafter. With hypodermic injection the amount is more nearly equal on 2 or 3 successive days, since the absorption may extend over 2 days.

8. Alkali-albumin, as well as muscle-proteids (from foreign species) are completely retained. An unconverted mixture of egg-albumen and sodium carbonate behaves like egg-albumen.

9. A small amount of proteid (less than 5%) is excreted unchanged by the fæces.

10. A variable proportion is excreted as non-coagulable proteid. The quantity of this is proportional to that of the coagulable proteid of the urine.

11. The rest undergoes complete metabolism to urea.

12. The total nitrogen excretion is increased beyond the amount of nitrogen introduced as albumen.

13. Starvation appears to cause an increase in the ratio of the urea to the total nitrogen of the urine.

14. The effects of intravenous injection of egg-albumen on circulation and respiration do not differ from those of an equivalent injection of the solvent. Albumen causes, however, a specific diuresis, beginning 50 minutes after the intravenous injection, and reaching its maximum in about 2 hours. It causes neither glycosuria nor hæmoglobinuria.

15. The injection of egg-albumen, alkaline egg-syntonin, or muscle extracts, causes in rabbits a rise of temperature of 1 to 2° C.

This begins in about an hour, usually reaches its maximum in from 6 to 8 hours, and then falls rapidly. It may in rare cases persist for several days. It is indifferent qualitatively whether the injection is made by the jugular or the ear-vein, hypodermically, or into the peritoneum. Even extremely small quantities injected into the ear-vein cause this rise. The fever does not cause histological alterations in any organ examined. The injection of normal salt solution may cause a rise, but this is much smaller.

16. The injection of egg-albumen causes but very slight histological changes. The kidneys are usually congested, especially in the cortex. The cells may be slightly cloudy. A slight degree of nephritis may occur, but this is not of such degree as to effect permanent lesions. The injection of muscle extracts may give rise to a more pronounced parenchymatous nephritis.

17. Urethane is fatal to rabbits in doses of 0.75 to 1.0 grm. per kilo. The symptoms consist mainly in a very marked fall of temperature, and in medullary paralysis. 0.5 grm. per kilo. lowers the temperature 2.3° C. Doses as small as 0.6 grm. per kilo cause very marked histological changes, consisting mainly in extensive granular and vacuolar degenerations of the hepatic epithelium, which are so acute as to be fully developed when death occurs in $1\frac{1}{2}$ hours after injection. Doses of 0.35 grm. per kilo. do not produce this change. Chloretone did not cause the degeneration, but is followed by congestion of the abdominal viscera.

18. Native egg-albumen, injected into the femoral vein of a dog, was followed in one case by a fatal ending with convulsions and coma, after several intervening cases of good health. Further experiments demonstrated that there is no toxicity inherent in fresh egg-albumen, nor can it be developed by brooding the eggs in the shell. The cause of the above fatal issue must therefore be sought in some extraneous toxic agent which contaminated the solution. Muscle-extracts were also devoid of toxicity. Alkali-albumin produces no changes beyond those which may be attributed to the free alkali contained therein.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE ACTION OF SAPONIN ON THE BLOOD CORPUSCLES AND PUS CORPUSCLES.

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I have shown in previous communications[1] that saponin produces a marked increase in the electrical conductivity of blood. The increase is quite as great in blood which has been fixed by formaldehyde as in fresh blood. It is, therefore, not dependent on the escape of the hæmoglobin from the corpuscles which saponin causes in unhardened blood, but not, of course, in blood treated with formaldehyde. I have demonstrated that no such effect is produced by saponin on blood-serum in the absence of the corpuscles. Its action on the conductivity of the blood may be explained in two ways: (1) It may increase the total conductance of the serum, (a) by causing the passage of electrolytes into it from the corpuscles without increasing its relative volume, or (b) by bringing about an increase in its volume, due to the passage of water out of the corpuscles and a consequent increase in the amount of dissociation of the electrolytes of the serum, or (c) by causing both water and electrolytes to pass out of the corpuscles.

(2) The saponin may increase the conductivity of the corpuscles without affecting that of the serum.

In the case of formaldehyde-hardened blood, where the action of saponin on the permeability of the corpuscles for electrolytes is most easily studied, since it is not complicated by the passage of the blood-pigment into the serum, it is not difficult to prove, by measuring the conductivity of the serum and sediment, respectively, after centrifugalization, that it is the conductivity of the corpuscles which is increased. If there is any increase in the conductivity of the serum,

[1] *Journ. of Physiology*, 1899, xxiv, p. 211, and 1901, xxvi, p. 470.

(a slight increase is perhaps indicated in some of the observations), it is small in comparison with the effect on the corpuscles. This is well seen in *Experiment I*,[2] in which the influence of saponin on the conductivity of unhardened blood is compared with that on blood fixed by formaldehyde. λ, as in the other experiments, is an abbreviation for $\lambda\ (5°) \times 10^8$. The conductivities are expressed in reciprocal ohms reduced to 5° C. The measurements were made by Kohlrausch's telephone method, the tube containing the blood being immersed in running water, the temperature of which did not vary more than 2° or 3° from 5° C. The saponin solution used was made by dissolving 3 grammes crude quillaia saponin in salt solution and adding salt solution up to 100 cc. The salt solution had in some experiments a strength of about 0.7 per cent, in others of about 1 per cent. Its conductivity was always carefully measured, as was also that of the saponin solution, which did not differ much from the conductivity of the salt solution. As a control, observations were also made on blood to which, instead of saponin solution, a similar amount of the salt solution was added.

It will be seen that in *Exp. I*, in the observation of April 18, the serum from the mixture of A (the formaldehyde blood) and saponin has λ 83.66, while the serum from the corresponding mixture of A with the sodium chloride solution has λ 78.48. A portion even of the small difference shown here is accounted for by the greater conductivity of the saponin than of the sodium chloride solution. On the other hand, for the sediment of corpuscles from the mixture of A and saponin, after prolonged centrifugalization, λ is 46.51 against 18.68 for the sediment of the control experiment with A and sodium chloride, and 14.76 for the sediment of A. The great difference between the sediments is certainly not to be explained by the presence of a greater quantity of serum between the corpuscles in the sediment of the saponin mixture than in the others, for the sediment was exceedingly compact and well-separated from the serum. There can be no doubt, therefore, that saponin does render the corpuscles better conductors. From this it necessarily follows

[2] The protocols of experiments are at the end of this article (p. 272).

that the "envelope" of the corpuscle is rendered permeable to ions to which it refuses passage before the action of the saponin, or more rapidly permeable to those which penetrate it only slowly and with difficulty. But the experiment does not enable us to say whether, in addition, the saponin produces an increased dissociation inside the corpuscle.

I desire to point this out clearly because in a recent paper on the increase of conductivity which is known to occur in dying muscle,[3] it is tacitly assumed that an increase of conductivity necessarily implies the formation of an increased number of ions in the muscle and is proportional to the increase in the number. Now while it is, of course, a perfectly familiar fact that when muscle dies a decomposition takes places which leads to the formation of substances capable of acting as conductors, we must not assume that this is the only way in which the conductivity is increased, until it is shown that no change in the resistance of the membranes through which the ions must pass is produced at death. The fact that the resistance of muscle is much greater in the transverse than in the longitudinal direction suggests very strongly that the resistance of the sarcolemma, and possibly also of whatever "envelopes" enclose the sarcostyles, is greater than that of the conducting liquids between the fibres or between the fibrils. And if those "membranes" are relatively impermeable to the electrolytes during the life of the tissue, as they should be if the proper osmotic relations are to be preserved, it is quite probable that their permeability, and therefore their resistance, becomes altered when the tissue dies. In this connection the fact mentioned in my previous paper, that perfectly fresh colored corpuscles are far less permeable to NH_4Cl than corpuscles which have stood for a few hours seems very suggestive.

I may mention here another observation which supports the conclusion that the envelopes of the corpuscles are altered by saponin. It was invariably found that when saponin was added at such an interval after the addition of formaldehyde that it no longer caused laking, the sediment of corpuscles settled far more slowly and less

[3] T. Kodis, *American Journ. of Physiology*, 1901, v, p. 267.

perfectly, was always more bulky in proportion to the liquid above it, and formed a less compact and tenacious mass on the bottom of the test-tube than the sediment from formaldehyde blood which had not been treated with saponin or to which NaCl solution had been added in the same proportion as the saponin solution. This refers to the sediment obtained when the blood was simply allowed to stand in a cold room and not to that separated by prolonged centrifugalization. It was always much easier to shake up into complete suspension in the serum the sediment of the formaldehyde blood which had been acted on by saponin; and the greater the amount of saponin solution added, the looser and more easily shaken up was the sediment. The same effect was observed, though it was not so pronounced, in blood to which NH_4Cl solution was added in such amount as to cause partial laking, and which was then treated with formaldehyde. The sediment was distinctly looser than when NaCl was substituted for NH_4Cl in this combination. The suggestion is that NH_4Cl alters the envelopes of the corpuscles in such a way that they are less liable to adhere firmly to each other when acted on by formaldehyde, and that saponin undoes or lessens the formaldehyde action which causes them to adhere. The fact that NH_4Cl penetrates the corpuscles much more readily than NaCl also shows that its relation to the envelope is different. In this experiment it is incidentally shown that the preference of the corpuscles for NH_4Cl as compared with NaCl is still evident 15 days after the addition of formaldehyde to the blood.

Some leucocytes are of course always present in the sediments whose conductivity is compared in the experiment, which, however, yields no information as to the action of saponin upon these. But some observations on pus, to be presently described, show that saponin affects the conductivity of pus corpuscles in very much the same way as that of the red corpuscles. Since, as I have previously shown, the action of saponin on the red corpuscles is essentially the same whether they are perfectly fresh or have stood for days, it seems highly probable that the conductivity of the leucocytes of blood is also increased by saponin, and in the same way, that is, by an increase of their permeability to the ions of the serum. The "envelope" of

the leucocyte (or at least of the pus corpuscle), however, differs from that of the colored corpuscle, as we shall see, in exhibiting no preference for NH_4Cl as compared with NaCl.

In *Experiment II* the effect of varying the quantity of saponin added to formaldehyde blood on its conductivity was investigated. Under λ the conductivities of the mixtures of formaldehyde blood and saponin are given, and in the column headed "λ of control" the conductivities of mixtures of the formaldehyde blood with similar quantities of the NaCl solution used in making the saponin solution. In the third column the differences between λ and λ of control are given, that is to say, the approximate amounts by which the conductivity of the blood has been increased by the action of the saponin, after deducting the increase due to the NaCl in the saponin solution. I shall speak of the numbers in this column as the true saponin effect.

The experiment shows that the addition of 1cc. of the saponin solution (the largest quantity used) to 5 cc. of the formaldehyde blood (A) causes practically the same true saponin effect as the addition of 0.4 cc. of the saponin solution to 5 cc. of A. In both cases, too, the full increase of conductivity is already developed at the time of the first measurement (a few minutes after mixture), and there is no further increase when the mixture is allowed to stand for many hours. When 0.2 cc. of the saponin solution is added to 5 cc. of A the true saponin effect ultimately becomes just as great as when 0.4 cc. or 1 cc. is added, but it has not reached its maximum 12 minutes after mixture. Eight hours after mixture the effect is complete, and there is no further increase after many hours. With 0.1 cc. of saponin solution to 5 cc. of A the true saponin effect is only 14.79, 20 minutes after mixture, as compared with 22.14, 19 minutes after the addition of 0.4 cc. of saponin solution to 5 cc. of A. But in the observation with 0.1 cc. of saponin solution the effect has not reached its maximum even 2 hours and 20 minutes after mixture, when it is 16.23. In 19 hours more it has further increased to 18.05. With 0.05 cc. of the saponin solution to 5 cc. of A the increase in the true saponin effect, as time goes on, is still more striking, the effect being only 5.39, 27 minutes after mixture, but 8.56, 4½ hours after mixture, and 9.31,

16 hours later. The maximum effect remains much less for this quantity of saponin than for 0.1 cc. or anything above it. With 0.02 cc. of the saponin solution (the smallest quantity employed) to 5 cc. of A the maximum effect is still less, but the relative increase between the first and last measurement is the greatest of all (from 0.74 to 3.33).

The action of saponin in laking the colored corpuscles has usually been ascribed to a solution or "corrosion" of some constituent of the stroma (or envelope). The fact that it greatly increases the conductivity of the formaldehyde-hardened corpuscles is an additional argument in favor of this view. It further shows that the substance on which saponin acts is not fixed by formaldehyde, within the longest period investigated (a fortnight), or at least is not altered in such a way as to become incapable of being attacked by saponin. This makes it highly probable that the substance is not a proteid. Ransom[4] has recently brought forward evidence that the substance on which saponin acts when it lakes the corpuscles is cholesterin. My results are compatible with this idea. But it seems certain that if the saponin produces its effect on the conductivity of formaldehyde blood by attacking the cholesterin in the corpuscles, the result is due not to any merely chemical reaction between the saponin and the cholesterin, but to a change of structure in the corpuscles which renders them more permeable to ions and perhaps in addition to an increased dissociation of electrolytes in the corpuscles. I have not hitherto found anything in the appearance of the corpuscles under the microscope or their behavior to stains which throws any light on the point of attack of the saponin.

In *Experiment III* I endeavored to determine whether the addition of saponin to yolk of egg, which is known to be rich in cholesterin (and lecithin) compounds would affect the conductivity at all in the same way as it affects the conductivity of blood. I found that it had no more influence on the yolk than on the white, and no greater effect on either than was due to the electrolytes contained in the saponin solution. On the other hand it was shown that there was a marked difference in the effect of dilution with water on the conduc-

[4] *Deutsche med. Wochensch.*, 1901, p. 194.

tivity of the white and the yolk respectively, the dilution causing a much smaller proportional diminution in the conductivity of the yolk than in that of the white. Thus in *Experiment IV* the conductivity of the yolk was 19.83 and that of the white 56.46. Dilution with 1 volume of water (Fig. 1) reduced the conductivity of the former to 17.76 and that of the latter to 33.98; while the addition of 3 volumes of water made the conductivity of the yolk 11.52 and that of the white 19.26. The conductivity of the undiluted yolk is always much less than that of the undiluted white, the ratio being usually about 1:3.

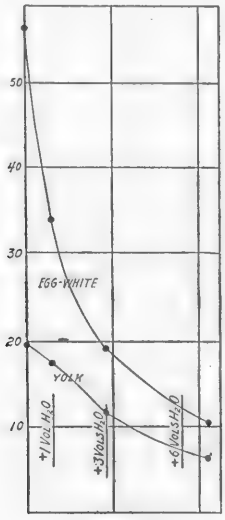

FIG. 1.

Curves showing the effect of dilution with water on the conductivity of yolk and of white of egg, plotted from Exp. IV. Conductivities laid off along vertical axis, and quantities of water added along horizontal axis.

The fact that saponin increases the conductivity of blood-corpuscles which have been fixed by formaldehyde suggests that its primary action on unhardened blood is the same, viz., an increase in the permeability of the envelope to the electrolytes of the serum or of the corpuscles themselves. The osmotic equilibrium would be thus

upset; some of the water of the serum would pass into the corpuscles; and when the increase of permeability reached a certain limit enough water might enter to cause of itself the discharge of the hæmoglobin. Saponin-laking would thus be reduced to a special form of water-laking, as Nolf[5] and Hédon[6] have suggested. My experiments, in so far as they demonstrate an actual increase of permeability for the electrolytes, are distinctly in favor of this hypothesis. Support is also lent to this view by the fact that an amount of saponin insufficient to produce laking may cause the blood to darken, the corpuscles swelling up without losing hæmoglobin. This change is not accompanied by the marked increase of conductivity which signalizes the actual onset of the laking process, although the conductivity may be somewhat increased.

Another fact, which, if it does not so directly support this theory of the saponin action, is at least consistent with it, is that the laking action of saponin and also its effect on the conductivity are much more easily produced at a temperature of 40° to 45° C. than at the ordinary temperature. A dose of saponin which may be ineffective at room temperature may cause complete laking and the characteristic increase of conductivity when the blood is heated to the temperature mentioned, and a dose which may be just capable of laking slowly at ordinary temperature will bring about rapid laking at 40° to 45°. Occasionally I have noticed, as in *Exp. V*, that a dose to which blood is refractory at 40° to 45°, becomes rapidly effective at 50° C. The phenomenon is different from heat-laking, for this cannot be obtained even by prolonged heating at such temperatures. The action seems to be simply the ordinary saponin action intensified by the increase of temperature, which may diminish the power of resistance of the envelope to the attack of the saponin and the penetration of the electrolytes.

A diminution in the resistance of the corpuscles to the action of saponin may be actually observed when blood is allowed to stand for a while before addition of the saponin. A given dose of saponin may

[5] *Ann. de l'Institut Pasteur*, 1900, p. 297.

[6] *Archives internat. de pharmacodynamie et de thérapie*, 1901, viii, p. 403.

then produce a greater increase of conductivity and more complete laking than the same dose produced in the fresh blood; and a dose which is insufficient to bring about complete laking, with the corresponding increase of conductivity, in fresh blood, may do so in stale blood.

With the view of throwing further light on the changes which occur in the serum and the corpuscles when unhardened blood is laked by saponin—a subject, as already remarked, less easy to study than the corresponding changes in formaldehyde blood—I have compared the conductivity and freezing-point of ordinary defibrinated blood and of formaldehyde blood before and after the addition of saponin. The same quantities were determined for the sera of the defibrinated blood, the formaldehyde blood and the mixture of the latter with saponin (*Experiment V*). It was found impracticable to separate the ghosts from the saponin-laked defibrinated blood by the centrifuge, and therefore in this case the freezing-point was measured directly on the laked blood, and the conductivity of the corpuscle-free liquid was not determined. But in *Experiment VI* the ghosts were completely separated from saponin-laked blood by filtration through a pot of porous clay, and the conductivity of the liquid measured.

In *Exp. V* from the percentage of serum in the defibrinated blood (estimated by the electrical method previously described by me[7]) and the actual Δ of the serum and saponin solution I have calculated the value of Δ for the extra-corpuscular liquid of the mixture of blood and saponin, on the assumption that no exchange of water or dissolved solids between the corpuscles and this liquid is caused by the saponin, or that, if there is any exchange, water accompanies the solids in such proportion as to leave the Δ of the extra-corpuscular liquid the same as if no exchange took place.

From the percentage of extra-corpuscular liquid[8] in the formaldehyde

[7] *Journ. of Physiology*, 1899, xxiv, p. 356.

[8] I have supposed for the purpose of calculating this percentage that the formaldehyde solution when added to blood simply mixes with the serum, without any alteration in the volume of the serum-formaldehyde mixture taking place through exchange with the corpuscles. Since the corpuscles preserve their normal size and shape this assumption is approximately correct. But although there is no notable movement of

blood A and the actual λ and Δ of this liquid and of the saponin solution I have, on the same assumption, calculated the value of λ and Δ for the extra-corpuscular liquid of the mixture of A and saponin. In calculating λ and Δ I have made no allowance for possible changes in the dissociation coefficient when mixture takes place. Comparison of the actual and calculated values of λ and Δ, of course, affords information as to the correctness of the assumption on which the calculation is based, and where it is erroneous indicates how it must be modified.

It will be seen that for the mixture of defibrinated blood and saponin the actual is distinctly greater than the calculated Δ. This is doubtless due in part to the escape of hæmoglobin into the serum. But the molecular weight of hæmoglobin is so great that even the solution of the whole of the hæmoglobin would not suffice to account for the difference. In *Experiment VI* the liquid obtained by filtering off the ghosts from saponin-laked blood had a conductivity about equal to that of the serum of the original defibrinated blood, although decidedly less than that of serum obtained from blood to which as much of the NaCl solution used in making the saponin solution had been added as was added of the saponin solution to the saponin blood. The deficiency in λ of the serum of the saponin-laked blood is easily explained by the depressing influence on the conductivity exerted by the dissolved hæmoglobin. Indeed the quantity of hæmoglobin in solution would cause a considerably greater deficiency than actually exists. So that it seems necessary to suppose that water has entered the corpuscles from the serum in greater proportion than electrolytes, or that if water has come out of the corpuscles it has been accompanied by electrolytes in such amount that, apart from the depressing influence of the hæmoglobin, the conductivity of the serum would have been increased. The latter alternative would also explain the excess of the actual over the calculated Δ in *Exp. V*, and it is this that we must accept. For although at the beginning of the saponin action water seems to enter the corpuscles and to cause them to swell, the process must be reversed with the discharge of the hæmoglobin, since in saponin-laked blood the ghosts constitute only a relatively small proportion of the total volume. We must, therefore, suppose that as the hæmoglobin escapes from the corpuscles, they also lose water and electrolytes.

water into or out of the corpuscles, much of the formaldehyde certainly enters them. This is well illustrated by the fact that for the serum from A the calculated is much greater than the actual Δ, while the actual λ is almost the same as the calculated. This shows that the difference between the actual and calculated Δ is due to the passage of non-electrolytes, and not of electrolytes into the corpuscles.

In the case of the extra-corpuscular liquid of the mixture of formaldehyde blood and saponin the difference between the actual and calculated Δ is too great to be explained by the passage even of all the saponin into the corpuscles. It is most easily accounted for by the supposition that saponin increases the permeability of the corpuscles for certain of the dissolved substances in the extra-corpuscular liquid. If any salts pass into the corpuscles, water must enter along with them, or other salts must escape from the corpuscles, since the actual λ of the corpuscle-free liquid (referred to as "top" in the tables) is practically the same as the calculated λ. The difference between the actual and calculated Δ must accordingly be due to the passage not of electrolytes but of non-electrolytes (formaldehyde) into the corpuscles. As has been shown in *Exp. I*, the marked increase of λ of the entire formaldehyde blood under the influence of saponin is due to an increase in the permeability of the corpuscles for ions. I have already given reasons for supposing that when ordinary (unhardened) blood is acted on by saponin the corpuscles become more permeable to the salts of the serum, or certain of them, and presumably to their ions. Whether this increase of permeability persists when the corpuscles have been reduced to ghosts by the escape of the hæmoglobin I am unable to say. The ghosts certainly remain worse conductors than the serum, as is shown by the fact that as the serum is separated from saponin-laked blood the conductivity of the residue sinks (see e. g. *Exp. VI*).

Another point of interest illustrated in *Exp. V*, and still better in *Exp. I*, is that the conductivity of a specimen of defibrinated blood treated first with formaldehyde and then after a while with saponin is not in general the same as the conductivity of another specimen of the same blood to which saponin is first added and then formaldehyde in the same amount as in the first observation. The difference, of course, depends on the presence of the corpuscles, and would not be seen if the electrolytes of the blood were all in simple solution. The combination blood + saponin + formaldehyde has in general a smaller conductivity than the combination blood + formaldehyde + saponin, especially when in the second combination blood which has been acted on so long by the formaldehyde that no laking takes place on the addition of saponin is employed. This supports the conclusion already drawn, that one of the causes of the smaller conductivity of the first combination is the depressing effect of the hæmoglobin on the conductivity of the laked blood. In addition, it is probable that a great part of the formaldehyde, which, as a non-conductor, depresses the conduc-

tivity of the extra-corpuscular liquid, is in the second combination fixed in the corpuscles.

THE BEHAVIOR OF PUS CELLS TO NH_4Cl, NaCl, SAPONIN AND WATER.

In order to see whether other cells, and especially dead cells, would exhibit any of the peculiarities studied in the colored corpuscles, I made some experiments with pus.

In *Experiment VII* it is shown that pus corpuscles, like colored blood-corpuscles, have a smaller conductivity than the serum of pus. The serum can be separated from the corpuscles by filtering the pus through porous clay, but more easily, and as completely, or nearly so, by filtering it through paper, putting the first portion of the filtrate back on the filter. I have had no success in obtaining the serum free from corpuscles by the centrifuge. On the average the conductivity of the pus serum is very much the same as that of blood serum, but sometimes it is considerably greater, especially, of course, if putrefaction is at all pronounced. I have preciously shown[9] that in putrefying liquids the conductivity increases and the freezing-point diminishes progressively up to a certain limit, as time goes on, owing to the conversion of proteids or other complex substances into simpler compounds, some of which are electrolytes. I have never obtained such small values for the conductivity of sediments rich in pus cells as for blood sediments, and, therefore, it would seem that pus corpuscles are more permeable to the ions of the serum than blood-corpuscles are. But, as is seen in *Exp. VII*, the conductivity of the sediment is much less than that of the serum.

When diluted with water (*Exp. VII* and Fig. 2) pus has a higher conductivity than a simple solution of electrolytes or blood-serum or egg-white diluted to the same extent, but a lower conductivity than correspondingly diluted yolk of egg (*Exp. IV* and Fig. 1) or defibrinated blood. This indicates that electrolytes may pass out of the corpuscles when pus is diluted with water. But in the absence of measurements of the conductivity and freezing point of the corpuscle-free liquid from the diluted pus one cannot make any positive statement as to this. For there are three other factors which would cause

[9] *Journal of Experimental Medicine*, 1899, iv, p. 235.

a relative increase of conductivity: increased dissociation of electrolytes in the serum, diminution of the depressing effect of the non-electrolytes, and increase in the average breadth of the conducting paths between the corpuscles.

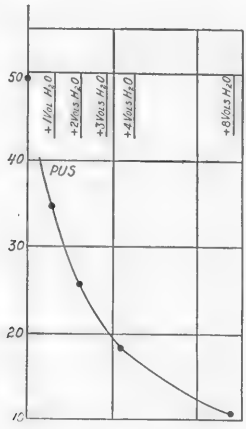

FIG. 2.

Curve showing the effect of dilution with water on the conductivity of pus, plotted from Exp. VII. Conductivities along vertical, quantities of water added along horizontal axis.

Heating pus to 69° C. (*Exp. VII*) and subsequent dilution failed to reveal any analogy between the behavior of pus corpuscles and that of blood corpuscles, the ratio between the conductivity of the pus which had been heated and that of the heated pus after dilution with its own volume of water being practically the same as the corresponding ratio for a specimen of unheated pus and for a sediment of pus. In *Exp. IX* it is shown that when pus is heated to 80° C. for 10 minutes its conductivity is increased. The increase is still greater when the pus is heated to 93° for 15 minutes, although the conductivity of the serum is diminished.

On the other hand, saponin, as is shown in *Experiment VIII*, increases the conductivity of pus just as it does the conductivity of blood. This is the case also when saponin is added to pus which has been treated with formaldehyde. The action is on the corpuscles, since the

addition of saponin to pus serum causes only the change of conductivity due to the electrolytes in the saponin solution (*Exp. IX*). When saponin is added to pus which has been heated to 93° C. (*Exp. IX*) the characteristic increase of conductivity is still produced, although the proteids of the corpuscles must have been coagulated. With the microscope the corpuscles, or at least great numbers of them, were seen to be still intact, and stained normally. These facts are in accordance with what has been already mentioned as to the action of saponin on formaldehyde blood, and suggest that in the case of pus also the constituent of the corpuscles on which the saponin acts is not of proteid nature.

At the same time it ought to be pointed out that the entire increase produced by saponin in the conductivity of the heated pus is not due to an action on the corpuscles; a portion of the increase is caused by some action of the saponin on the serum. This is proved in *Experiment IX*, where the behavior to saponin of the serum of unheated pus is compared with that of the serum of the same pus after heating to 93°. In the case of the serum of the unheated pus the saponin, as already stated, produces only the small increase of conductivity corresponding to the electrolytes in the saponin solution. The increase in the conductivity of the serum of the heated pus is too great to be entirely accounted for in this way. I have no results as yet which would enable us to decide whether the action of the saponin on the serum of the heated pus causes the granules present in it (some of which may have escaped from corpuscles either partially or wholly broken down by the heating) to become better conductors, or whether the increase in conductivity is due to the action of the saponin on dissolved substances. It cannot be due to any action on intact corpuscles in the serum, for it was shown by the microscope that none such were present.

There is no evidence (*Exp. VIII*) that pus corpuscles take up NH_4Cl in preference to NaCl either in the case of normal pus or of pus hardened by formaldehyde.

SUMMARY.

1. The increase of conductivity produced by saponin in formaldehyde-hardened blood is due to an increase in the conductivity of the

corpuscles (increased permeability of the corpuscles to ions) and not, mainly at any rate, to the liberation of electrolytes from the corpuscles and a consequent increase in the conductivity of the serum. The increase in the permeability of the corpuscles is probably caused by a "corrosive," dissolving, or emulsifying action of the saponin on some non-proteid constituent of the envelope or stroma.

2. In the first stage of the action of saponin on blood (not fixed by formaldehyde) there seems also to be an increase in the permeability of the corpuscles for ions, even before any hæmoglobin has been liberated. The liberation of the hæmoglobin may be secondary to this, owing to the entrance of water consequent on the disturbance of osmotic equilibrium.

3. Heating the blood to 40° to 45° C. intensifies the laking action of saponin, so that a dose insufficient to cause laking at ordinary temperature may do so when the blood is heated to the temperature mentioned.

4. Pus corpuscles, like red blood corpuscles, are worse conductors than the serum in which they are suspended. Unlike blood corpuscles, they show no preference for NH_4Cl as compared with NaCl. On the other hand, the conductivity of pus is increased by the action of saponin, just as is the case with blood, and apparently very much in the same way, that is to say, by an action on the corpuscles and not on the serum. The fixing of the pus corpuscles by formaldehyde does not hinder this action of saponin.

PROTOCOLS OF EXPERIMENTS.

EXP. I.

April 17. At 10.20 A. M. obtained defibrinated blood from a bitch. At 3.50 P. M. added to 150 cc. of the defibrinated blood 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution. The mixture is called A. For the formaldehyde solution $\lambda=64.18$; for the saponin solution $\lambda=83.94$; for the NaCl used in making the saponin and formaldehyde solutions $\lambda=78.97$. Percentage of serum in the defibrinated blood calculated by the electrical method, 51.5.

Time.	Defibrinated blood.	λ
April 17, P. M.		
8.30	Defibrinated blood	30.18
7.17	20 cc. defib. blood + 1.6 cc. saponin.	
7.23		34.63
7.45	Not laked, only somewhat darkened; heated in bath at 44° to 45° along with A + saponin. A + sapo. lakes far sooner and more completely than defib. blood + saponin.	
8.10	Defib. blood + saponin (well laked)	37.95
8.14	To the mixture of defib. blood and saponin added 10 cc. formald. sol.	
8.45	This mixture	43.71
	(After standing 18 hours)	44.55
April 18, P. M.		
3.05	Defibrinated blood	28.87
	Serum from clot	84.22
2.55	20 cc. blood + 1.6 cc. saponin.	
3.17	Only somewhat darkened; not at all laked.	
4.25	Not laked, although dark.	
6.15	Now laked	43.11
6.20	Added to this mixture of defib. blood and saponin 10 cc. formald. sol.	
6.37	This mixture	47.56
	(After standing 13 hrs., perfectly laked and still thin)	46.86
	Bottom* (after centrif. 20 min.)	47.47
3.17	20 cc. blood + 1.6 cc. of the NaCl sol. used in making the saponin sol.	
6.05		32.09
	(After standing 13½ hrs.)	29.55

Time.	Formaldehyde blood A.	λ
April 17, 8.37 P.M.	A	37.01
7.38	30 cc. A + 1.6 cc. saponin sol.	
7.45	Considerably darkened.	
8.23	Well laked	56.33
	(After standing 18 hrs. is thick and viscid)	53.26
April 18, P. M.		
3.33	30 cc. A + 1.6 cc. saponin sol.	
6.20	Not laked	58.41
	(After standing 13 hrs. is still thin and not laked)	59.79
	Top (after centrif. for 50 min.)	83.66
	Bottom	46.51
	A + as much of the NaCl sol. used in making the sapo. solution as was added of the saponin sol.	
	(After standing 22 hrs.)	41.90
	Top (after centrif. for 20 min)	78.48
	Bottom	18.68
	Top of A (after centrif. for 50 m.)	79.72
	Bottom	14.76
April 19, 8.01 P.M.	15 cc. A + 1.2 cc. saponin sol.	
8.05		58.95
	(After standing 18 hrs.)	61.38
	Top (after centrif.)	80.22
	Bottom	51.42
8.12	10 cc. A + 20 cc. water.	
8.17		23.02
	(After standing 17 hrs.)	23.78
	Top	30.11
	Bottom	13.60
	Bottom (after centrif.)	7.52
8.25	A	35.96
May 2. A. M.		
10.49	A	38.53
11.00	5 cc. A + 0.4 cc. saponin sol.†	
11.19		66.53
P. M.		
7.53		66.19
	(19 hours later)	66.36
A. M.		
11.00	5 cc. A + 0.4 cc. of the NaCl sol.‡ used in making the saponin sol.	
11.11		44.09
11.20	5 cc. A + 5 cc. NH_4Cl §	
	(After standing 24 hours	74.34
	Top	103.47
11.20	5 cc. A + 5 cc. NaCl ‡	
	(After standing 24 hours)	80.22
	Top	111.18

* There is very little separation. The ghosts cannot be separated by the centrifuge but can be completely separated by filtration through paper. After a time both the filtrate and the sediment on the filter set into a very firm jelly.

† For this saponin solution $\lambda = 139.30$.
‡ For the NaCl solution $\lambda = 140.07$.
§ For the NH_4Cl solution $\lambda = 143.10$.

EXP. II.

To show effect of different quantities of saponin on the conductivity of formaldehyde blood. Formaldehyde blood A (from Exp. V) was used. On April 17, 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution was added to 150 cc. of bitch's defibrinated blood. For the saponin solution $\lambda=139.30$. For the NaCl solution used in making the saponin solution $\lambda=140.07$.

Time.		λ	λ of control.	Difference bet. λ and λ of control.
May 2,				
10.49 A. M.	A	38.53		
11.00	5 cc. A+0.4 cc. saponin.			
11.19		66.53	44.09	22.44
7.53 P. M.		66.19	.....	22.10
	(19 hours later).....	66.36	.....	22.27
11.50 A. M.	5 cc. A+0.2 cc. saponin.			
12.02		62.44	41.35	21.09
8.01 P. M.		63.85	.....	22.50
	(19 hours later).....	63.85	.....	22.50
3.13 P. M.	5 cc. A+0.1 cc. saponin.			
3.33		54.52	39.73	14.79
8.08		55.96	.....	16.23
	(19 hours later).....	57.78	.....	18.05
3.50	5 cc. A+0.05 cc. saponin.			
4.17		43.86	38.47	5.39
8.15		47.03	.....	8.56
	(16 hours later).....	48.19	38.88	9.31
3.50	5 cc. A+1 cc. saponin.			
4.23		73.91	51.11	22.80
8.23		72.84	.....	21.73
	(16 hours later).....	72.84	.....	21.73
4.35	5 cc. A+0.02 cc. saponin.			
4.50		39.80	39.06	0.74
8.30		40.95	.....	1.89
	(15½ hours later).....	42.39	.....	3.33

EXP. III.

White and yolk of two fresh hen's eggs, beaten up separately and strained through muslin. For the saponin solution $\lambda=140.83$; for the NaCl solution used in making it, $\lambda=141.62$.

	λ		λ
White.....	59.09	Yolk.....	21.45
5 cc. white + 0.8 cc. saponin solution.....	69.26	5 cc. yolk+0.8 cc. saponin solution.....	35.06
5 cc. white + 0.81 cc.* of the NaCl sol. used in making the saponin sol.....	69.07	5 cc. yolk+0.81 cc.* of the NaCl sol. used in making the saponin sol.....	35.36

* .01 cc. too much was added by mistake.

EXP. IV.

White and yolk of hen's egg beaten up separately.

	λ	Ratio of λ of undiluted to λ of diluted.		λ	Ratio of λ of undiluted to λ of diluted.
White.....	56.46		Yolk.....	19.83	
+1 vol. water..	33.98	1.66	+1 vol. water..	17.76	1.11
+3 " " ..	19.26	2.93	+3 " " ..	11.52	1.72
+7 " " ..	10.41	5.42	+7 " " ..	6.78	2.92

EXP. V.

To dog's defibrinated blood, two hours after it was drawn, one-half of its volume of a 4 per cent solution of formaldehyde in a 1 per cent NaCl solution was added. Call the mixture A. The measurements in the table were begun 18 hours later. The animal had been used for intravenous injection of glycerine extract of suprarenal capsule one hour before the blood was obtained. For the formaldehyde solution $\lambda=111.18$; $\Delta=3.755$. For the NaCl solution $\lambda=139.30$; $\Delta=0.732$. For the saponin solution $\lambda=134.84$; $\Delta=0.851$. The proportion of serum in the defibrinated blood as calculated by the electrical method is 60.6 per cent. The proportion of serum (extracorpuscular liquid) in A, calculated on the assumption that the addition of the formaldehyde caused neither loss nor gain of water by the corpuscles, is 73.7 per cent.

DEFIBRINATED BLOOD.						FORMALDEHYDE BLOOD (A).					
Time.		λ actual.	λ calculated.	Δ actual.	Δ calculated.	Time.		λ actual.	λ calculated.	Δ actual.	Δ calculated.
P. M.						P. M.					
1.18	Serum from defibrinated blood..	85.93	....	0.761		1.32	Serum from A (practically free from blood pigment).....	96.75	97.3	1.686	2.11
4.22	Defibrinated blood.....	38.47				2.21	A	61.53			
2.59	To 20 cc. of defib. blood added 1.6 cc. saponin solution.					2.27	To 25 cc. of A added 1.33 cc. of the saponin sol.				
6.10	No laking. Added 0.4 cc. more of the saponin solution.					6.10	No laking. Added 0.4 cc. of the saponin sol.				
6.35	Not laked. Heated to 44° for 15 min. Darkens somewhat, but is not laked.					6.35	Not laked. Heated to 44° for 15 min. No change.				
6.50	Added 0.4 cc. more of the sapo. sol. Left at 45° for 16 min.; is darkened but not laked.					6.50	Added 0.4 cc. more of the saponin solution, and left in bath at 45° for 16 min.				
7.25	Added 0.4 cc. more of the saponin solution.					7.25	Added 0.4 cc. more of the saponin solution.				
7.35	Not laked. Heated to 50° for 10 min.; lakes almost at once.					7.35	Not laked. Heated to 50° for 10 min.; although darker than A, it is not laked.				
7.54	Seems perfectly laked.....	56.58									
	(After standing 23 hrs. more)...	66.36	....	0.799	.778	8.05	Not laked	80.22			
	Serum from mixture of blood and saponin	....	95.10				(After standing 15½ hrs. more).	81.25			
8.35	To 11.4 cc. of the mixture of blood and saponin added 5 cc. of the formaldehyde solution.						Top (after centrifugalization).	102.22	101.3	1.427	1.585
9.03	(Quite thick, but can still be poured out).....	75.00	....	....	1.97						
	In 4 min. more it set completely into a jelly, so that Δ could not be determined.										
	Top.....	....	101.5	....	1.97						

EXP. VI.

Dog's defibrinated blood. For the saponin solution $\lambda=140.83$; for the NaCl solution used in making it $\lambda=141.62$.

	λ
The defibrinated blood	33.75
Serum from clot (free from haemoglobin)	88.33
To 50 cc. of defibrinated blood added 4 cc. of saponin solution (dark and laked in 5 minutes)	68.51
Filtered the saponin blood several times through paper, and centrifugalized the filtrate.	
Filtrate (contains many ghosts and leucocytes)	71.01
Filtered this filtrate through a pot of porous clay.* (Now free from corpuscles)	88.64
Residue containing the ghosts and leucocytes but also much serum	67.78
To 25 cc. of defibrinated blood added 2 cc. of the NaCl solution used in making the saponin solution. This mixture	38.47
Serum from this mixture (free from corpuscles)	98.26

* It filters much more slowly than water-laked blood, obtained by adding 2 volumes water to the same defibrinated blood, and filtered under the same pressure.

EXP. VII.

	λ	Ratio of λ of pus to λ of diluted pus.
Pus from tubercular hip joint	49.51	
+1 vol. water	34.12	1.45
+2 "	25.66	1.92
+3½ "	18.56	2.66
+8 "	10.39	4.75
Clear serum from pus (filtered through clay)	86.52	
Fresh pus obtained on another occasion from the same hip joint	57.61	
Clear serum (filtered through paper)	77.52	
Sediment	56.58	
Sediment + 1 vol. water	39.45	1.43
" +3 vols. water	22.04	2.56
Another specimen of pus	49.56	
Clear serum from it (filtered through paper)	91.30	
Sediment	38.55	
Another specimen of pus	49.94	
Heated to 69° C. (became thicker than original pus)	50.49	
Heated pus + 1 vol. water	34.87	1.44

EXP. VIII.

Pus from a case of empyema obtained May 7. On May 8, at 8 P. M., added to 50 cc. of the pus 20 cc. of a 4 per cent solution of formaldehyde in NaCl solution. Call the mixture A. For the formaldehyde solution $\lambda=123.66$. For the saponin sol. $\lambda=140.83$; for the NaCl solution $\lambda=141.62$; for the NH_4Cl solution $\lambda=143.90$.

Time.	Pus.	λ	Time.	Formaldehyde pus (A).	λ
May 8, P. M.			May 9, P. M.		
8.09	Pus	46.51	4.26	A	66.19
	Serum separated by filtration	109.27		Serum from A	93.54
7.36	10 cc. pus + 10 cc. NH_4Cl.		4.40	10 cc. of A + 10 cc. NH_4Cl.	
8.19		89.58	7.19		101.81
	(After standing 12 hours more)	89.26	4.40	10 cc. of A + 10 cc. NaCl.	
	Top	117.91	7.25		101.00
7.37	10 cc. pus + 10 cc. NaCl.		4.49	5 cc. of A + 0.4 cc. saponin.	
8.25		89.26	7.32		78.48
	(After standing 20 hours more)	88.64	4.49	5 cc. of A + 0.4 cc. of the NaCl solution used in making the saponin sol.	
	Top	116.28			
7.41	10 cc. pus + 0.8 cc. saponin.				
8.31		59.65	7.40		70.03
	(After standing 11½ hrs. more)	61.98	4.52	5 cc. of A + 10 cc. of water.	
7.42	10 cc. pus + 0.8 cc. of the NaCl sol. used in making the saponin solution.		7.48		32.67
				(After standing 16 hrs.) Top	38.82
			May 14, A. M.		
8.37		52.27	11.47	5 cc. of A + 0.4 cc. of saponin.	
7.45	10 cc. pus + 20 cc. water.		P. M.		
8.49		29.34	12.07		80.47
9.10	10 cc. pus + 1.6 cc. saponin.		A. M.		
	(After standing 10½ hrs.)	74.34	11.47	5 cc. of A + 0.4 cc. of the NaCl solution used in making the saponin sol.	
9.10	10 cc. pus + 1.6 cc. of the NaCl sol. used in making the saponin solution.				
	(After standing 10½ hrs.)	56.08	12.00		71.41

EXP. IX.

Same pus as that used in Exp. VIII obtained on May 7. The solutions of NH_4Cl, and NaCl, saponin are the same as those used in Exp. VIII.

Time.		λ	λ of control *
May 14,			
3.00 P. M.	Pus heated to 80° C. for 10 minutes	56.71	
3.10	5 cc. of the heated pus + 0.4 cc. saponin.		
3.28		63.69	61.23
3.48	The original pus (not heated)	52.81	
4.00	Pus heated to 93° C. for 10 minutes	61.23	
4.20	5 cc. of this heated pus + 0.4 cc. saponin.		
4.41		68.14	64.67
May 15,			
2.30 P. M.	Clear serum from original pus (filtered through paper, entirely free from pus corpuscles)	109.27	
2.42	5 cc. of this serum + 0.4 cc. saponin.		
2.59		111.67	111.67
7.21	Serum from pus which was heated to 93° (separated by centrifuge. Very few pus corpuscles in it, but a good many granules)	95.66	
7.16	5 cc. of this serum + 0.4 cc. saponin.		
7.45		105.63	101.81
May 16,			
10.53 A. M.	Serum from pus which was heated to 93° (separated by filtration through paper. Entirely free from pus corpuscles. Not many granules can be seen till methylene blue is added, when a good many appear, many of which are cocci)	93.54	
11.10	5 cc. of this serum + 0.4 cc. saponin.		
11.26		106.51	98.26

* In this column are given the conductivities of pus or serum of pus to which instead of saponin solution the corresponding amount of the NaCl solution used in making the saponin solution was added as a control.

SNAKE VENOM IN RELATION TO HÆMOLYSIS, BACTERIOLYSIS, AND TOXICITY.

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(*From the Pathological Laboratory of the University of Pennsylvania.*)

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INTRODUCTION. GENERAL CONSIDERATIONS CONCERNING HÆMOLYSIS AND BACTERIOLYSIS.

[I have long desired that the action of venoms upon blood should be further examined. I finally indicated in a series of propositions the direction I wished the inquiry to take. Starting from these the following very satisfactory study has been made by Professor Flexner and Dr. Noguchi. My own share in it, although so limited, I mention with satisfaction.—S. WEIR MITCHELL.]

The following research, which is presented at this time in abstract, was conducted under a grant from the Bache fund of the National Academy of Sciences. It forms the first instalment of a new study

of venoms upon which we have been engaged during a year past and which, it is hoped, will be continued during another year or longer.

While these studies are still incomplete, the data here given have been worked out in detail and may therefore be accepted as final. On account of the large number of tables which will be given in the final publication, and the many drawings necessary to illustrate properly the text, more or less delay in bringing out the full work will be inevitable. But inasmuch as the results of the studies form an integral part of the work on hæmolysis and bacteriolysis, which is now attracting so much attention among bacteriologists and pathologists, and as they contain certain facts of fundamental importance bearing on the theory of these phenomena, it seems best not to delay publication until the entire series of researches shall have been completed.

At present we shall not give the full bibliography. Since the fundamental studies of Weir Mitchell and his collaborators,[1] the effects of venom upon the blood and the nervous system of animals have been generally recognized. The rapid putrefaction which sets in after poisoning with venom was also explained by Welch and Ewing's[2] observations on the loss of bactericidal power of the serum of such poisoned animals. The close relationship of the poison with certain toxins of bacteria and of higher plants was shown by the discovery of Sewall,[3] of Calmette[4] and of Fraser[5] that animals could be immunized from the effects of venom and that they yielded an active antitoxin. That the poison of venom is not simple, but that it consists of a complex of constituents of a proteid nature was proven by Mitchell and Reichert. The time, therefore, seemed ripe for a further study of the physiological effects of venom upon the blood, upon bacterial life, and upon tissues, in the light of the recent studies upon various kinds of immunity.

For the purpose of these studies dried venom has been employed.

[1] *Smithsonian Contrib. to Knowledge*, 1860, xii, and 1886, No. 647.
[2] *Lancet*, 1894, i, p. 1236.
[3] *Journal of Physiology*, 1887, viii, p. 203.
[4] *Ann. de l'Institut Pasteur*, 1894, viii, p. 275.
[5] *British Med. Journ.*, 1895, i, p. 1309.

Fortunately several kinds were available—through the kindness of Prof. Reichert, that of the rattlesnake (*Crotalus adamanteus*); of Dr. Joseph McFarland, that of the water mocassin (*Ancistrodon piscivorus*) and of the cobra (*Naja tripudians*), and of Messrs. Mulford & Co., that of the copperhead (*Ancistrodon contortrix*). We wish to express to these gentlemen our great appreciation of their kindness.

Before presenting the matter of our studies, it seems best to preface what we have to say with a brief statement of some of the facts and views relating to the phenomena of hæmolysis and bacteriolysis. In this preface we shall not distribute credits for the work or the views here embodied, inasmuch as this will be done in the complete publication. Only so much will be said as is necessary for an understanding of the experimental data relating to venom which are to follow.

By hæmolysis is meant solution of the blood-corpuscles. The term is usually applied to solution of the red corpuscles, but the white cells are also subject to a similar solution. It is, therefore, correct to speak of hæmolysis when both kinds of cells, erythrolysis when the red cells only, and leucolysis when the white cells alone are dissolved. In this solution of the red cells, which is the type of hæmolysis, the hæmoglobin is separated from the stroma of the corpuscles. The separation of hæmoglobin by hypotonic solutions and through the action of destructive chemical substances is not considered in this article. Hæmolysis as here employed refers to such separation through the action of complex agents derived from living plants or animals. This form is distinguished as biologic hæmolysis. Of all such agents the most active are found in the blood plasma or serum of alien animal species. Others are the products of cellular activity, such as venom, certain toxic products of bacterial growth, as tetanolysin, staphylotoxin, etc., and still others are yielded by some of the higher plants, as crotin from *Croton tiglium*.

The most familiar examples of hæmolysis are supplied by the effects of the transfusion of animal blood into man. It was early discovered that the practice was dangerous for the reason that the red corpuscles of the host were dissolved by the foreign blood. This effect was quickly seen to be due to the serum of the alien blood and it was observed to take place with equal readiness *in vitro*. The blood of animals

also is hæmolysed by foreign sera—the red corpuscles of the rabbit, for example, being dissolved readily by dog's serum. Some sera have very high dissolving power, the most active thus far known being that obtained from the eel, which is correspondingly toxic. After admixture of the corpuscles and foreign serum, solution does not occur immediately. The corpuscles first run together, become clumped, or, as we now denominate the effect, agglutinated. The dissolving effect of venom upon corpuscles is also preceded by a similar agglutination, as was first shown by Mitchell and Stewart.[6]

There is much similarity in the phenomena of agglutination and of lysis as observed in blood-corpuscles with appearances seen in connection with bacteria. The Gruber-Widal reaction of agglutination, which has served so well in the diagnosis of typhoid fever and other bacterial infectious diseases, is of a similar nature. Moreover, under certain conditions solution of the agglutinated bacteria may also occur, when bacteriolysis more or less analogous to hæmolysis results. The well-known Pfeiffer phenomenon, in which cholera spirilla undergo disintegration and solution in the peritoneal cavity of the immunized guinea-pig is the classical example of bacteriolysis.

The studies of the past two or three years upon the allied phenomena of bacteriolysis and of hæmolysis have not only demonstrated their fundamental similarity, but provided chemical explanations of the processes involved. Pfeiffer observed that the serum of immune animals caused agglutination only of the bacterial species used for immunization; he believed that for complete solution of the bacteria the mixture must be brought into the living body, and for this purpose he chose the peritoneum of the guinea-pig. Somewhat later Metchnikoff and Bordet discovered that the same effect could be produced *in vitro* by the addition to the immune serum of a small quantity of peritoneal exudate, or even of the fresh serum of an animal. From this experiment it could be inferred that for agglutination of bacteria certain bodies were required which were in the immune serum; but for solution still other substances contained in part in fresh serum were requisite. That this second substance might also be present in the original immune serum could now be shown; and it was also demonstrated that it is of a very labile nature and quickly disappears spontaneously. The addition of fresh serum or exudate restores it. It may be destroyed by raising the temperature of the fluid to 56° C.

A great advance in our knowledge of cytolysis was made when it was

[6] *Trans. College of Physicians of Philadelphia*, 1897, 3. s., xix, p. 105.

discovered that immunization to blood and body cells gives rise to the production of lysins. Just as in bacterial immunization lysins for the special bacteria employed are yielded, so also are evolved analogous substances for red and white blood cells, for epithelial cells, spermatozoa, etc. Indeed the number and variety of lysins that can be produced experimentally are limited only by the number and variety of animal cells available. Blood cells of one animal may be used to produce a lysin in the body of another animal of the same species—isolysin; in another species—heterolysin; and success has in rare instances followed the re-injection of withdrawn blood through which autolysins have been produced. Nor is the production of lysins the only result of the injection of cellular structures. Preceding the solution, clumping of the cells takes place from which it may be concluded that agglutinins are also formed.

The factors required for producing solution of cells are similar to those for causing solution of bacteria under like conditions. Only when the lytic serum is very fresh will solution be effected; the addition, however, of peritoneal exudate or fresh normal serum to immune serum which has lost the solvent property, suffices to restore it.

A consideration of the preceding facts shows that the agglutinating principle is distinct from the dissolving one. This consideration also indicates that more than one body is necessary to bring about solution either of bacteria or of animal cells.

An analysis of the phenomena suggests that at least two substances are requisite. One is stable and contained in the immune sera (whether for bacteria or animal cells); the other is labile, and while originally contained in the immune sera, it is lost spontaneously. This latter substance is a normal constituent of the lymph and blood plasma, for it can be restored by the addition of these fluids.

Experiments conducted in a very convincing way by Ehrlich and Morgenroth indicate: (1) that a special principle is concerned in agglutination—the so-called agglutinin; and (2) that two principles are concerned in lysis. These principles are different in origin. One—that which is stable—is the product of immunization, and, on account of certain combining properties possessed by it, they call it the 'intermediary body.'[7] The other is normally present in the body juices but

[7] Ehrlich has recently suggested the name 'ceptor,' in place of 'intermediary body.' According to the manner of action he distinguishes 'uniceptors' and 'amboceptors.' Bordet calls this body 'substance sensibilisatrice;' Metchnikoff 'fixator;' P. Mueller, 'copula.'

is easily destroyed by heat and tends to disappear spontaneously when the fluids are removed from the body. This latter principle, on account of the complemental nature of its action, they propose to call the 'complement.'[8]

There is conclusive experimental evidence that, although the intermediary body unites first with the cells—bacterial, blood cells, etc.—this substance by itself cannot bring about solution. But after the union of this intermediary body with the cells the complement is capable of being brought into action, through this intermediation, so that solution takes place. The union of intermediate body and cells is conceived to take place through certain combining (haptophore) groups present in the cells and in the intermediary substance; while the complement is linked through similar combining (haptophore) groups possessed by the intermediary body and itself. The intermediary body, therefore, carries two sets of combining or haptophore groups: one for the cells and the other for the complement (complementophilic group). The complement possesses in addition to such a corresponding haptophore group, another group which exhibits fermentative properties (zymotoxic or toxophore group), through the action of which solution of cells takes place.

This conception of lysis applies not solely to that produced by immunization, but the same factors are believed to be operative in the solution of blood cells or of bacteria by normal blood sera. Here also an intermediary body and a complement are brought into action; the only difference being that, in the one case, the intermediary body is produced through artificial immunization, and in the other, it is present normally, but whether because of some insidious and unperceived change, similar to but slighter than artificial immunization, is not known.

It would carry us too far afield to give in detail the elaborate views of Ehrlich and his co-workers as to the origin of the intermediary bodies. Suffice it to say that they are the products of immunization by bacterial or other cells, and are believed by them to be yielded by certain constituents of cellular protoplasm within the body, designated as 'lateral chains,' which through their haptophore groups are capable of combining with the haptophore groups of protoplasmic constituents of the bacteria or the cells used for the immunization. When dealing with the toxic constitution of venom more will be said concerning this aspect of the subject.

[8] This body is called 'alexin' by Bordet, and probably agrees in part with the body of the same name described by Buchner Metchnikoff calls it 'cytase.'

VENOM-AGGLUTINATION.

For the study of agglutination all the available varieties of venom were employed. Several kinds of animal blood—from the dog, rabbit, guinea-pig, sheep, ox, pig, *Necturus*, and frog—were tested. Either the blood was defibrinated or the specimen consisted of the corpuscles separated by centrifugalization and washed six times, as a rule, in 0.8% normal saline solution (washed corpuscles). The different venoms showed slight differences only in the degree of agglutination, with the exceptions of the action on the blood of *Necturus* and the frog, which are not affected in weak solutions but show agglutination in stronger solutions (2%).

The usual method consisted in dissolving in normal saline solution dried venom in strengths ranging from 0.01% to 10%—the last that of *Crotalus adamanteus*. The phenomena of agglutination appear rapidly in favorable solutions, while in very weak solutions a delay of some minutes up to one hour may be noted. The corpuscles which come together thus slowly do not show the great modification of shape that is characteristic of those that fuse more completely and quickly. In a general way it may be said that the several varieties of dried venom with which we experimented gave, when employed in the strength of 0.5%, what are to be regarded as maximal agglutinations for mammalian corpuscles. Active agglutination still takes place in 0.2% solutions, while weaker ones either produce no change at all or show an imperfect fusion.

The morphological changes need not be described here as these have been fully dealt with by Mitchell and Stewart.[9]

The value of the use of washed corpuscles comes especially from the fact that the succession of lytic phenomena is eliminated. Agglutination, therefore, may be studied purely. For this purpose a 5% solution of the corpuscles in normal salt solution was employed. That complete agglutination has no effect upon subsequent solution (lysis) of the corpuscles will be shown when treating of the latter phenomena. On the other hand, distinct differences in susceptibility

[9] Loc. cit.

to agglutination have been observed. Of the mammalian blood thus far employed the red corpuscles of the rabbit may be said to be highly susceptible, while those of the guinea-pig, dog, sheep, swine, and ox were less responsive in about the order given.

The use of defibrinated blood permitted observation upon the succession of phenomena of agglutination and hæmolysis. In general it may be said that the first effect of the venom is the production of agglutination to be followed by solution after a variable interval, depending on the kind and strength of the venom and on the temperature. There are, however, notable exceptions in that the range of lytic activity of venoms is greater than that of the agglutinating property. Very weak solutions of venom which no longer cause agglutination may still be capable of producing solution.

Moreover, on account of the action of two sets of factors in defibrinated blood—one tending to produce agglutination, and the other solution of the red corpuscles—the degree of agglutination is here less marked than in the washed cells where no lysis occurs. This difference is explained by the fact that a part of the corpuscles go into solution before agglutination can take place; and hence the extent of precipitation and fusion varies inversely with the susceptibility to lysins. As a consequence, dog's corpuscles which are more easily hæmolysed by venom than any others of the animal bloods tested by us show the least degree of agglutination. The rapidity of agglutination in any case is not affected by ordinary temperatures. Hence a low temperature (0° C.) permits, even in defibrinated blood, the separation of the phenomena of lysis and those of agglutination. At this temperature defibrinated dog's blood behaves as do the washed corpuscles, the amount of precipitation being, therefore, greater than at the temperature of the room or of the thermostat.

The agglutinating power of venoms is destroyed by temperatures of 75° to 80° C. maintained for thirty minutes.

VENOM-HÆMOLYSIS.

Unless mention is especially made the same group of animals was employed in these studies as in the foregoing. The venom solutions

varied from 5% to 0.0001%, depending upon the source of the corpuscles and the variety of venom. The venoms differ in hæmolytic power as follows: cobra most active; water mocassin, copperhead, rattlesnake, in less degree in the order named. A similar variation in susceptibility to the reaction could be distinguished in the different mammalian bloods employed. Thus dog's blood was most quickly and easily hæmolysed and it responded to the greatest dilutions, while the corpuscles of the ox were the least susceptible. The intermediate animals were in about the following order: sheep, guinea-pig, pig, and rabbit. On the other hand, the blood of *Necturus* is acted upon slightly after longer periods, and frog's blood almost not at all after equally long periods.

The extent of variation in the response of the different bloods is considerable. Very strong solutions of venom (5%) are needed to cause hæmolysis of corpuscles of the ox, but such great strengths are without action upon rabbit's corpuscles, although they are still capable of producing rapid solution of dog's or sheep's corpuscles. Taken altogether solutions averaging 0.2 per cent of venom have proven the most favorable for bringing out the hæmolytic effect upon blood generally.

Defibrinated Blood.—In making the tests with defibrinated blood uniform mixtures were employed. In all experiments 5 per cent of blood was added to the venom solutions, and the mixtures were kept at temperatures varying from 36° to 37° C.

The differences in activity of venoms are shown by the following series in which minimal active solutions are given for several kinds of blood.

Dog's blood hæmolysed by solutions of *Crotalus* venom 0.001%; copperhead 0.0005%; water-mocassin 0.0002%; cobra 0.0001%

Sheep's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.0005%.

Guinea-pig's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.001%.

Swine's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.001%.

Rabbit's blood hæmolysed by solutions of *Crotalus* venom 0.005%; copperhead 0.002%.

Rabbit's blood hæmolysed by solutions of water-mocassin venom 0.002%; cobra 0.001%.

Ox's blood hæmolysed by solutions of *Crotalus* venom 0.05%; copperhead 0.02%.

Effect of Heat upon Hæmolytic Power of Venoms.—Temperatures of 75° to 80° C. for thirty minutes have no effect upon the hæmolytic action of any kind of venom. From 90° to 96° C. *Crotalus* venom in solution suffers a moderate reduction in hæmolytic power, while the remaining venoms are entirely unaffected at these temperatures. After heating to 100 C. for fifteen minutes the dissolving power of cobra, mocassin, and copperhead venom in solution is slightly reduced.

Effect of Venoms upon Washed Blood-Corpuscles.—In no instance were the washed blood-corpuscles hæmolysed by venom. Agglutination occurs as already described. But if the separated serum is restored to each of the several kinds of blood-corpuscles treated with venom, lysis takes place.

Certain important differences may be noted. Thus if the quantity of serum added to rabbit's corpuscles exceeds the normal, quicker and more complete solution occurs than is noted in defibrinated blood. A similar but less striking action may be observed with the blood of the guinea-pig. This action depends upon the union first effected between the red corpuscles and the intermediary body of venom, the latter later combining with the introduced complement of the serum so that solution takes place. That this is the mechanism of the action the succeeding experiments prove. But before taking them up another set of phenomena must be briefly considered.

Rabbit's and guinea-pig's washed blood-corpuscles (hereafter termed washed corpuscles) are quickly dissolved by fresh dog's serum. Dog's corpuscles are but little acted upon by fresh rabbit's serum and not at all by guinea-pig's serum. Rabbit's and guinea-pig's sera are about equally hæmolytic for each other's corpuscles. *Necturus'* serum is highly hæmolytic for rabbit's, dog's, and guinea-pig's corpuscles. Frog's serum is less destructive for the mammalian cor-

puscles mentioned than that of *Necturus*, but is still very active. *Necturus*' serum and frog's serum are slightly and equally active on each other's corpuscles.

This dissolving action is not noted at freezing temperatures. If, therefore, washed corpuscles which have been treated with serum for thirty minutes at zero temperature are separated by centrifugalization or precipitation, the complement in the serum is unaffected, while the intermediary body will be found to have been removed from the serum by the corpuscles. In this way the complement for a particular species of corpuscles, free from the intermediary body, can be obtained. The addition of such complement-containing serum to venomized washed corpuscles of the same species brings about hæmolysis, while the addition of fresh washed corpuscles to the treated serum, from which the intermediary body for them has been removed, is unattended by solution.

The action of complements, freed from any intermediary body by this means, upon venomized corpuscles of different species has also been studied. The results are of interest. The procedure is as follows: Let us suppose that it is desired to test the effects of rabbit's serum upon venomized dog's corpuscles. The rabbit's serum is first treated with washed dog's corpuscles in the cold to withdraw all combining intermediary bodies; the clear serum having been separated is now added to the venomized corpuscles, when solution of a slow and limited nature occurs, thus showing that there exists in the dog's corpuscles a limited number of receptors capable when venomized of uniting with rabbit's complement.

Controls for these experiments were made in the following manner: Any serum treated with alien corpuscles at zero temperature and then separated from the corpuscles by centrifugalization has become inactive for this kind of fresh washed corpuscles (tested for dog's, guinea-pig's, rabbit's, and *Necturus*' corpuscles). However, if to the inactive mixture the same variety of serum heated to 58° C. is added (this serum containing intermediary body but without complement) solution takes place.

The degree of interaction of different species of sera minus inter-

mediary bodies upon different species of venomized washed corpuscles is shown by the following series, in which copperhead venom is used throughout. 1 cc. of 0.2% solution of venom is mixed with 0.05 cc. of washed corpuscles and 0.5 cc. of complement, with these results:

Dog's corpuscles and rabbit's complement = slow and imperfect hæmolysis.

Dog's corpuscles and guinea-pig's complement = slight hæmolysis, more marked than preceding.

Rabbit's corpuscles and dog's complement = rapid and imperfect hæmolysis.

Rabbit's corpuscles and guinea-pig's complement = weak and imperfect hæmolysis.

Guinea-pig's corpuscles and dog's complement = rapid and imperfect hæmolysis.

Guinea-pig's corpuscles and rabbit's complement = slow and imperfect hæmolysis.

Dog's corpuscles and *Necturus*' complement = slight and imperfect hæmolysis.

Guinea-pig's corpuscles and *Necturus*' complement = no action.

Rabbit's corpuscles and *Necturus*' complement = no action.

Venom solution treated with dog's, rabbit's, and guinea-pig's washed corpuscles in succession gives up to each a part of its intermediary bodies. No one kind of corpuscle is capable of fixing the entire content of intermediary bodies. The supernatant fluid probably contains still other intermediary bodies capable of fixation by still other corpuscles. If to the several kinds of venomized corpuscles here mentioned different complements are added, then, as shown in the foregoing series, lysis will or will not take place, depending on the nature of the complement employed; but so long as the complement is foreign to the corpuscles it never causes complete solution.

From these results the following conclusions are warranted: (1) Venom contains several or many intermediary bodies. (2) These bodies show specific affinities for certain complements. In addition to this there is evidence that the many susceptible corpuscles contain, besides specific haptophore groups for intermediary bodies, certain common haptophore groups, which are shared, perhaps, by all vulnerable corpuscles.

Combined Action of Venom and Ricin. Relation of Agglutination and Hæmolysis.—Agglutination produced by venom does not affect lysis. But on the other hand when lysis takes place quickly, agglutination may fail or may appear imperfectly. The principles causing the two phenomena are distinct in the manner of combination and in action. That the agglutinative and the lytic principles are different is now proven; and there is evidence that they act upon different constituents of the red cells. Thus, if ricin, a strong agglutinator, is permitted to act upon red corpuscles for periods under thirty minutes, then upon the addition of venom lysis ensues in about the average time and proceeds normally. If, however, the ricin has acted for two or more hours, then solution by venom still takes place, but the stroma of the corpuscles remains in the bottom of the test-tube as a white conglutinated mass. From this it appears that agglutination brings about a kind of coagulation of the stroma, from which, through the action of the hæmolysin in venom, hæmoglobin has been released. Ricin is without action upon venom itself, and conversely ricin is equally unaffected by venom.

VENOM-LEUCOLYSIS.

In the blood snake-venom causes destruction of the leucocytes as well as of the red cells. In order to ensure a more accurate study of its action upon the white blood-cells, these were obtained in larger quantities and without admixture of red cells by injecting positively chemotactic substances into the pleural and peritoneal cavities of the rabbit. For this purpose cultures of *B. megatherium* killed by heat were injected into the pleural cavity and sterile bouillon into the peritoneum.

From eighteen to twenty-four hours after the introduction of *B. megatherium* into the pleural cavity, fluid rich in leucocytes may be withdrawn by means of capillary tubes without sacrificing the animal. The same procedure employed twenty-four hours after the injection of bouillon yielded a fluid less rich in leucocytes, and consequently this second method was not extensively employed.

The leucocytes in the fluids thus obtained could be separated ac-

cording to size and granulation into lymphocytes (20 to 25% of the total leucocytes), finely granular, medium-sized cells (60%), larger non-granular cells (3%), still larger irregular and coarsely granular cells (4%), and others again somewhat smaller but showing very coarse granules (6%). The susceptibility to the destructive effects of venom varied somewhat for the different cells. Those of the largest size with coarse granules are most quickly affected; next to these come the finely granular varieties, the lymphocytes showing the least injury of all.

In studying the changes taking place in the leucocytes under the influence of venom a warm stage (37° C.) was used, the edges of the cover glasses having first been sealed with vaseline. The venom solutions varied from 10% to 0.002%. The weakest effective solution was that of cobra venom (0.002%), whereas in the case of the rattlesnake and of the mocassin, 0.002% and 0.005% respectively, caused definite changes.

Only the granular cells showed motility. Weak active solutions are without immediate effect on motion but begin to manifest an inhibiting action after about one hour, the controls being still motile at the end of two hours or longer. After the motility ceases, the cells in general, except the lymphocytes, show increased granulation due to the appearance of coarser and more numerous granules in the protoplasm, the nuclei coincidently becoming more distinct. After six hours the majority of the largest granular cells have already disintegrated, the nuclei having been liberated. After twenty-four hours most of the medium-sized granular cells have suffered disintegration, while the lymphocytes show but slight and inconspicuous changes. Stronger solutions, varying from 0.2% to 10%, cause instant cessation of motility and rapid agglutination without distinction of variety of cells. Within five to thirty minutes thereafter dissolution sets in, affecting first the largest, then medium-sized cells, and finally the small lymphocytes.

There are variations in the activities of the several venoms and in the completeness of solution of the cells. Rattlesnake venom is far less active than that of the cobra. Thus in 2% solutions cobra venom

causes complete solution in thirty minutes while that of the rattlesnake requires two hours to bring about the same result.

The effects upon washed leucocytes differ from those described in that venom solutions cause agglutination, but with the production of only very slight lysis.

Are the Hæmolysins (Erythrolysins) Identical with Leucolysins?—Copperhead venom (1 mg. in 4 cc. of normal saline) was treated with washed rabbit's red corpuscles at the thermostat temperature for thirty minutes, until the supernatant fluid after centrifugalization was without action upon defibrinated rabbit's blood. This solution when brought into contact with leucocytic fluid was without agglutinating action upon the cells while still causing their solution in about thirty minutes. On the other hand, the parallel experiment in which venom solution was treated with washed leucocytes yielded a fluid still active for defibrinated blood.

The conclusions from these experiments are as follows:

(1) Venom contains principles which are agglutinating and dissolving for white blood-corpuscles.

(2) The agglutinating principles may be identical for both white and red cells.

(3) The dissolving principle for leucocytes is distinct from that for red cells.

(4) In order that solution of venomized leucocytes shall occur a complement-containing fluid is required.

(5) The several varieties of white cells of the rabbit's blood show different susceptibilities to the action of venom.

VENOM-TOXICITY.

For the study of the toxic principles copperhead venom was chiefly employed. The animal selected for these experiments was the guinea-pig. The method of procedure was the following: We first determined for the particular sample of venom to be used the minimal lethal dose. This was found to be 0.3 mg. for a guinea-pig weighing from 250 to 300 grammes, death resulting within 24 hours. A dose of 0.6 mg. caused death in from two to three hours, and of 0.9 mg. in from 30 to 45 minutes.

Our special purpose was the determination of the existence of neutralizing substances for venom in the tissues of the body. The following tissues and organs were employed: brain, liver, spleen, kidney, voluntary muscle, adrenal gland, and blood. For this purpose the tissues were first washed in tepid, sterile, normal saline and a weighed quantity (2 grammes) was taken. This was triturated in a sterile mortar and mixed in test tubes with three times the minimal lethal dose (M. L. D.) of venom.

The mixture was now placed in the thermostat where it remained for one hour. It was then centrifugalized and the supernatant fluid was injected into the guinea-pigs. In the course of the experiment the original volume of the venom-solution suffered a loss amounting on an average to one-third of the whole volume. There should, therefore, after this subtraction remain behind at least twice the minimal lethal dose ($2 \times$ M. L. D.).

In the case of the blood, washed corpuscles were employed in excess, the other steps remaining the same as in previous experiments.

The results of these experiments are as follows:

Control	Dead in 45 minutes.
Brain	" 19 hours.
Blood	" 3 hours and 50 minutes.
Adrenals	" 2 hours and 35 minutes.
Spleen	" 2 hours and 10 minutes.
Liver	" 1 hour and 30 minutes.
Kidney	" 1 hour and 55 minutes.
Muscles	" 1 hour and 30 minutes.

In the next experiment 2 M. L. D. of venom were employed. The control died in five hours. The animals receiving the venom solution treated with the organs, etc., reacted as follows:

Brain	Survived.
Blood	Dead in 28 hours.
Liver	" 19 hours.

Relation of Neurotoxic to Hæmolytic Principle.—That these two principles are distinct is rendered probable by the effects of washed red-corpuscles and of brain tissue respectively upon the toxicity of

venom. Blood-corpuscles remove little or perhaps none of the toxic constituent that brain cells do away with *in toto*. The proof of difference can, however, be brought in another way: Four M. L. D. of venom were treated with an excess of red corpuscles and the supernatant fluid was injected into a guinea-pig; death ensued in 30 minutes. The same quantity of venom having been treated with four grammes of brain emulsion, the supernatant fluid injected into a guinea-pig caused death in 48 hours. The experiments in which 9 M. L. D. of venom were used resulted, (1) in the case of the blood in death in 25 minutes; and (2) in the case of the brain (7 grammes of brain having been employed) in 25 hours.

The supernatant fluid from the brain emulsion was strongly agglutinating and hæmolytic for defibrinated blood, while that from the washed corpuscles had lost all these properties. The supernatant fluid from the brain emulsion, when treated with an excess of washed corpuscles, re-centrifugalized and the fluid then injected, is non-toxic for guinea-pigs.

These experiments show: (1) that the neurotoxic and the hæmolytic principles are physiologically distinct; (2) that while the chief toxic constituent unites with the nerve cells, in multiple M. L. D. from which the neurotoxic principle has been removed a quantity of hæmolysin may be contained sufficient to bring about fatal intoxication.

These results are in keeping with the views expressed by Ehrlich and supported by Wassermann and Takaki's experiments on the fixative power of cells for certain groups of toxic substances. They tend, therefore, to support the hypothetical considerations of Ehrlich on which he bases his well-known lateral-chain theory of immunity. Expressed in the terms of this hypothesis brain cells may be said to contain the receptors for the neurotoxic constituent of venom, whereas blood cells furnish the receptors for the hæmolytic principle; these receptors are distinct and specific, and are not contained to any considerable amount, and perhaps not at all, in the liver and kidney cells and, if at all, in small quantity only in adrenal cells. Walter Myers found that the adrenal cortex possessed a very feeble combining power

for cobra venom, most marked in mammals (sheep) in which the cortex of the organ is well developed. He also observed little effect from the adrenal of the guinea-pig.

EFFECTS OF VENOM UPON BACTERICIDAL PROPERTIES OF BLOOD SERUM.[10]

The animals employed were the dog, rabbit, and *Necturus;* the venoms belonged to the cobra, moccasin, copperhead, and rattlesnake, and the bacteria were *B. typhi*, *B. coli*, and *B. anthracis.* The method consisted in (1) introducing venom into the animal and drawing the blood from the femoral artery into sterile Nuttall bulbs; (2) permitting the blood from the normal animals to enter Nuttall bulbs in which the venom solution was contained; (3) admixture of the venom in sterile solution (heated for 4 days to 56°-60° C.) with separated serum.

The bactericidal effects of the normal sera were first established. Rabbit's serum is highly destructive for *B. typhi* and *B. anthracis* and least for *B. coli.* Dog's serum is highly destructive for *B. typhi. Necturus'* serum is also very destructive to *B. typhi* and *B. coli.* It is without marked effect on *B. anthracis.*

Serum Venomized in vivo.—Cobra venom was most active. Blood from rabbits which had received 10 mg., taken 57 minutes after the injection, showed great loss of bactericidal properties.

Experiment LXXI.—1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1841	4254	7152
After 1 hour	767	3125	4130
" 3 hours	2488	13460	4320
" 6 "	Innumerable	Innumerable	17280
" 24 "	"	"	Innumerable

The controls for this experiment showed complete destruction of all bacteria, or, as in a few experiments, of all except *B. coli,* which

[10] In order to determine whether any effect is produced on the growth of bacteria by the presence of venom in culture media, varying small quantities of venom were added to nutrient agar-agar. The bacteria—*B. anthracis, B. coli, and B. typhi*—grown upon these tubes underwent rapid involutions and exhibited marked plasmolysis, as compared with control tubes of the same organism.

showed considerable diminution until after six hours when increase began.

Experiment LXX.—30 mg. rattlesnake venom injected; blood taken after 45 minutes. 1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	838	9940	5750
1 hour	756	6240	3654
3 hours	2930	Increase	6219
6 "	Increase	Innumerable	About 10000
24 "	Innumerable	Innumerable	Innumerable

Blood mixed with Venom in vitro.—In this series rabbits only were employed. The venom solutions were placed in Nuttall's bulbs and the blood from the femoral artery was permitted to stream into them. In each experiment 6 mg. of venom were mixed with 20 to 30 cc. of blood. Coagulation was very slow or completely inhibited and the serum was obtained when necessary by centrifugalization. It invariably contained hæmoglobin.

Experiment LXXIII.—1 cc. of venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1644	4580	4085
1 hour	2080	10760	4870
3 hours	18930	149740	24730
6 "	Innumerable	Innumerable	Innumerable

Experiment LXXII.—1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	736	3720	1275
1 hour	407	2340	920
3 hours	860	22210	8720
6 "	5220	Innumerable	Innumerable
24 "	Innumerable		

This series of experiments may be open to the criticism that the increased nutritive value of the serum because of the hæmoglobin present may have been the cause of the effects noted; as a control,

therefore, peptone was added to the serum in the proportion of 6 mg. of peptone to 20 cc. of serum.

Experiment LXXX.—Peptone added to rabbit's blood: 1 cc. employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1043	5120	7430
1 hour	193	2240	1534
3 hours	87	578	71
6 "	22	520	262
24 "	0	Innumerable	About 20000

From this experiment it follows that improvement in nutritive value reduces bactericidal effect but in far less amount than is noted in the parallel case of venom.

That the nutritive change is unimportant is shown by the first experiments, in which the poisoning was done *in vivo* and also by those to follow in which venom was added directly to the separated serum.

Experiment LXXXV.—Rabbit's serum 1 cc. with rattlesnake venom 1 mg.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	745	3990	5430
1 hour	594	4667	3136
3 hours	4486	12120	43430
6 "	About 100000	Innumerable	About 200000
24 "	Innumerable		Innumerable

Experiment LXXXII.—Dog's serum 1 cc. with copperhead venom.

	Venom 6 mg. *B. typhi*	Venom 1 mg. *B. typhi*	Control; norm. ser. *B. typhi*
Immediate	8860	3572	5808
1 hour	21120	6525	584
3 hours	65250	14950	184
6 "	Innumerable	Innumerable	92
24 "			0

In order to determine the least quantity of venom required to remove the bactericidal properties of the serum varying quantities of copperhead venom were employed. Dog's serum was chosen with *B. typhi*. In each case 1 cc. of serum was used.

Experiment LXXXVII (a).—1 cc. dog's serum and varying amounts of copperhead venom.

	Venom 1/2 mg.	1/5 mg.	1/10 mg.	1/20 mg.	1/50 mg.
Immediate	5970	3070	4290	4940	3350
1 hour	6240	3960	1830	2620	920
3 hours	12810	10000	6730	1350	593
6 "	Innumerable	100000	13140	172	15
24 "		Innumerable	Innumerable	About 10000	0

From this it may be concluded that the specimen of venom employed by us destroys the bactericidal properties of dog's serum when added in the proportion of 1/20 mg. of venom to 1 cc. of serum; and that 1/50 mg. in the same quantity of serum is practically without action.

In view of these positive results the partial inaction of venom upon *Necturus* serum is both remarkable and important.

Experiment CII.—1 cc. *Necturus* serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	4215	5127	8350
1 hour	6950	453	233
3 hours	18340	84	12
6 "	50170	4	0
24 "	Innumerable	0	

Experiment CIII.—Same as above and copperhead venom 6 mg.

	B. coli	*B. typhi*
Immediate	5237	3846
1 hour	615	394
3 hours	95	11
6 "	180	2
24 "	2950	3105

Control: *Necturus* serum 1 cc. + peptone 6 mg.

	B. coli	*B. typhi*
Immediate	9270	4530
1 hour	3810	577
3 hours	632	92
6 "	78	2
24 "	1850	927

Experiment CIV.—*Necturus* serum 1 cc. + copperhead venom 1 mg.

	B. coli	*B. typhi*
Immediate	6240	7360
1 hour	1170	833
3 hours	583	96
6 "	25	13
24 "	0	0

It may be remarked that *Necturus* is highly refractory to venom. An animal weighing 250 grammes received without effect 0.05 grm. venom, equivalent to 160 M. L. D. for the guinea-pig.

The effect of heat upon venom in relation to its action upon the bactericidal properties is of interest. For this purpose cobra, rattlesnake, moccasin, and copperhead venoms were studied. Temperatures varying from 75° to 90° C. were employed, and the heated venoms were mixed with the streaming blood in Nuttall's bulbs and with the separated serum. The venom was kept at the lower temperature (75°) for 30 and the higher (90°) for 15 minutes.

The heated venom acts just as the unheated except in the case of rattlesnake venom, the effect of which is somewhat diminished at the higher temperature, 90° C.

The Mechanism of the Action of Venom upon Serum.—That the bactericidal action of serum depends upon the intermediary body and complement seems established. That the influence of venom upon this property does not depend upon changes in the nutritive value of the serum the foregoing experiments prove conclusively. It is therefore possible that venom acts injuriously upon the intermediary body or the complement or upon both bodies at the same time. The complement is destroyed by heating serum to 56°-58° C.—a temperature which does not affect the intermediary body.

Experiment XCVIII.—To test effect of venom on the intermediary body.

(1) Copperhead venom 1/20 mg.; rabbit's serum 1 cc., and rabbit's serum heated to 58° C., 1 cc. (2) Control; rabbit's serum heated to 56° C.

	(1) *B. typhi*	(2) *B. typhi*
Immediate	4990	5320
1 hour	5800	7800
3 hours	18840	12400
6 "	Innumerable	Innumerable

Experiment XCIX.—To test effect on the intermediary body.

(1) Copperhead venom 1/10 mg.; dog's serum 1 cc.; dog's serum heated to 56°, 1 cc. (2) Control; serum heated to 56° C.

	(1) *B. typhi*	(2) *B. typhi*
Immediate	2270	3440
1 hour	2680	3950
3 hours	71950	12800
6 "	Innumerable	Innumerable

From these experiments the conclusion can be drawn that venom is without action upon the intermediary body contained in dog's and rabbit's serum.

The next experiment was to determine whether any action was exerted by venom upon the complements of these sera. For the purpose of obtaining the serum-complement free from the intermediary body, the rabbit was treated with dog's serum heated to 56° C. In this way the anti-intermediary-body was obtained, which, when heated to 56° C. (to remove rabbit's complement) and added to fresh dog's serum neutralized the action of the latter upon rabbit's corpuscles. From this it could be concluded that the intermediary body of the dog's serum was neutralized by the anti-intermediary-body contained in the immunized rabbit's serum, leaving behind the pure dog's complement in the fluid.

Experiment XCIX (a).—Action on complement.

(1) Fresh dog's serum 1 cc., and copperhead venom 1/10 mg., and dog's complement 1 cc.[11] (2) Control, dog's serum 1 cc., and 1/10 mg. venom.

[11] To obtain these complements fresh dog's or rabbit's serum was treated with rabbit's or guinea-pig's serum containing the anti-intermediary-body which was heated

	(1) *B. typhi*	(2) *B. typhi*
Immediate	5270	4360
1 hour	930	5980
3 hours	28	25410
6 "	15	Innumerable
24 "	0	

A similar experiment in which anti-intermediary-body for rabbit's serum was produced in the guinea-pig gave practically identical results except that when 1/10 mg. of venom was employed the neutralizing effect of this quantity on the complement was also exerted upon the second quantity of complement added.

Experiment XCVIII (a).—Copperhead venom; fresh rabbit's serum 1 cc., and rabbit's complement 1 cc.

	B. typhi 1/10 mg. venom	*B. typhi* 1/20 mg. venom
Immediate	4590	3280
1 hour	3740	1360
3 hours	1850	730
6 "	4900	110
24 "	Innumerable	0

From the experiments under the present heading the following conclusions are warranted:

(1) All venoms when used in suitable quantities destroy the bactericidal properties of many normal blood sera.

(2) The manner of this destruction consists in the fixation of the serum-complements by the venoms.

(3) Venoms have no action upon the intermediary bodies of serum.

(4) If the venom is incapable of uniting with the serum-complements (*Necturus*) then the original bactericidal properties remain unaffected by the presence of the venom.

EFFECTS OF ANTIVENIN ON HÆMOLYSIS AND BACTERIOLYSIS.

Through the kindness of Dr. McFarland we secured a small vial of Calmette's antivenin. This was used to test the restraining action

upon venom hæmolysis and venom anti-bacteriolysis. The antivenin was first proven to be non-hæmolytic for rabbit's corpuscles and to improve slightly the nutritive value of fresh rabbit's serum.

Erythrolysis by cobra venom on rabbit's corpuscles is prevented if neutralization by antivenin is effected. Thus 2 mg. of venom + 1 cc. of antivenin is still lytic although action is retarded; 1.5 mg. of venom + 1 cc. of antivenin caused slight hæmolysis after 24 hours, while 1 mg. + 1 cc. was without action.

In the case of rattlesnake venom 1 cc. of antivenin neutralized 3 mg. of the poison.

Leucolysis was affected in approximately the same degree as in the case of erythrolysis.

The effect on bacteriolysis is equally marked. When cobra or rattlesnake venom is treated with a neutralizing quantity of antivenin and fresh serum is added the resulting fluid behaves in a manner similar to that of the control mixture of normal fresh serum and antivenin.

Antivenin, therefore, neutralizes venom and removes both the hæmolytic and the anti-bacteriolytic actions.

ON A COCCIDIUM (*KLOSSIELLA MURIS*, GEN. ET SPEC. NOV.) PARASITIC IN THE RENAL EPITHELIUM OF THE MOUSE.

By THEOBALD SMITH, M. D., and HERBERT P. JOHNSON, Ph. D.

(*From the Laboratory of Comparative Pathology, Medical Department of Harvard University*).

Plates XXI–XXIII.

In 1889 one of us[1] published a preliminary description of a polysporous coccidium found in the epithelium of the convoluted tubules of the mouse's kidney. Several stages of the parasite were seen but the scantiness of the material left many gaps in the life-cycle. During investigations recently made to determine the mode of transmission of the Sarcosporidium of the mouse one of us[2] found a considerable number of gray mice, caught in the animal room connected with this laboratory, whose kidneys were abundantly invaded by this coccidium. This favorable opportunity of examining more thoroughly into the life-history of this sporozoon was utilized, and, as a result, we are able to add materially to the knowledge of this cell parasite. In the following pages some details given in the first paper will be repeated to avoid obscurity in the descriptions as well as to aid those to whom the first paper is inaccessible.

Thus far we have found only adult mice infected. The invaded kidneys are a trifle enlarged and the surface is faintly uneven. The most characteristic feature is a very delicate mottling of the whole surface with minute, barely visible, grayish specks. This appearance of the kidneys may be considered almost diagnostic of the presence of the parasite.

[1] Smith. *Journal of Comparative Medicine and Surgery*, 1889, x, p. 211.

[2] Smith. *Journal of Experimental Medicine*, 1901, vi, p. 1.

METHODS.

To study the parasite in the fresh state a bit of the cortex is teased in physiological salt solution on a slide and gently spread by pressure on the cover-glass. This simple procedure enables us to gain an insight into that portion of the life-cycle of *Klossiella* through which it passes in the renal tubules, for, as a rule, all stages are present in the same heavily-infected kidney. Even the nuclei can be made visible by treatment with aceto-methyl green.[3] Schneider's aceto-carmine has been found less satisfactory as a nuclear stain for fresh material. Kidneys intended for sectioning were halved lengthwise, fixed and hardened in Zenker's fluid for 24 hours, washed in running water for the same period, and then passed through 50 and 70 to 95 per cent alcohol. Other fixing agents were tried (Flemming's fluid, aceto-sublimate, and picro-acetic), but they possessed no advantages over Zenker's fluid. The tissue was embedded in paraffine, melting at 51°-53° C., and cut in ribbons. Sections as thick as 10-15 μ were occasionally useful, as each section included more of the parasite, which, owing to its minuteness, is not easily studied in serial sections.

The most useful stains have been ferric-alum hæmatoxylin and Mayer's hæmatate of ammonia (hæmalum), the latter used alone or followed by Van Gieson's picro-acid fuchsin. The latter, however, can only be used after prolonged staining with the hæmalum, otherwise it will completely mask the nuclear stain. Fine results are obtained by staining with eosin followed by Unna's polychrome methylene blue. Ferric-alum hæmatoxylin gives by far the clearest and sharpest picture of any stain, but certain delicate differentiations of the cytoplasm are best brought out by prolonged staining (24 hours or longer) with hæmalum followed by picro-acid fuchsin.

THE DEVELOPMENTAL STAGES OF *KLOSSIELLA MURIS*.

An adequate description of the various stages found in the kidney will necessitate a certain order beginning with the earliest stage we have seen. Before proceeding to such a detailed account it may be well to summarize very briefly the life-cycle as we now know it.

[3] Glacial acetic, 5 pts., distilled water 95 pts., methyl green to saturation.

The youngest parasite is a minute nucleated cell (sporont) found within the cytoplasm of the epithelial cells of the convoluted tubules. This cell enlarges, the nucleus divides into 12 or more daughter-nuclei which are next found near the now slightly-lobed or mammillated periphery of the parasite (mother-sporoblast). The cytoplasm now separates into 12 or more spheres with each nucleus as a centre (sporoblast stage). These spheres secrete a membrane (spore stage), and the contents of each spore break up into about 30 nucleated, falciform bodies (sporozoites). Each parasite thus produces in the kidney at least 360 individuals. In severe infections the convoluted tubules are frequently found blocked with these spores. The sporozoites are not set free in the kidneys, as intact spores are found in the bladder. The further life-history of this parasite remains conjectural, although it is highly probable that the spores enter the body with the food and that the sporozoites are set free in the digestive tract.

To this cycle must be added another body of different morphology, which is parasitic in the epithelium of Bowman's capsule and whose significance will be discussed later on.

A. THE PARASITE OF THE CONVOLUTED TUBULES (SPOROGONIC CYCLE).

1. *The earliest stage (sporont).*—In fresh teased tissue the invaded cells frequently become detached and in these the sporonts are best studied. The host cell is enlarged, pear-shaped, and the parasite lies within it, not in contact with the cytoplasm, but in a vacuole (Plate XXII, Fig. 3). Not infrequently 2 sporonts are contained in the same cell; each then lies within its own vacuole (Plate XXII, Fig. 5). The sporont measures 8 μ to 11.5 μ in diameter.

A very striking feature of the sporont is the storage of reserve food material in the form of spherical, highly refractive granules, to the number of 10 to 20 (Plate XXII, Figs. 3 and 4). These are usually accompanied by smaller granules of the same optical appearance and chemical properties (Plate XXII, Fig. 4). The large granules measure 1.5 μ in diameter, the smaller, 1 μ or less. The former disappear at or towards the close of the sporont stage; the latter per-

sist and even increase in number long after the daughter-sporoblasts are formed (Plate XXIII, Fig. 14), but there is no indication that the larger granules break up to form the small ones. The granules of both sorts are doubtless of a proteid nature and come under the head of "plastin granules" described by various students of the Sporozoa. They are highly resistant toward all the chemical agents with which we have tested them. It is in fact possible with strong mineral acids and alkalies to dissolve all the rest of the cell and still leave the granules intact. The large granules stain a vivid yellowish-green with aceto-methyl green; the smaller granules remain unstained. Schneider's aceto-carmine fails to stain either kind of granule. Iodine in a solution of potassium iodide gives them a yellowish tint, which remains unchanged on the addition of dilute sulphuric acid. They are not blackened by osmic acid, and are not dissolved by absolute alcohol. We conclude that they are neither of a starchy nor of a fatty nature but a highly resistant proteid, such as is known to occur widely among Sporozoa.[4]

In fixed and stained preparations the shrinkage has usually obliterated the vacuole in which the sporont lies. The latter appears as if embedded in the cytoplasm of the host (Plate XXII, Figs. 5 and 7). Concerning the mode of invasion of the host cell by the sporozoite we are unable to give any facts. The parasite, when first seen, was already spherical in form and 7 μ in diameter. At this stage the invaded cells are considerably increased in size and have assumed the characteristic flask shape (Plate XXII, Fig. 7), with the parasite near the broad distal end of the flask. As the parasite grows the host cell must continue to grow. It protrudes far into the lumen of the tubule, almost occluding it, and it may even cause a local enlargement of the lumen (Plate XXII, Fig. 7). The peduncle by which the cell maintains its attachment to the basement membrane and through which it derives nourishment becomes more and more slender and assumes a very characteristic granular appearance (Plate XXII, Fig. 7, and Plate XXIII, Fig. 13). This portion stains darkly with hæmatoxylin

[4] Bütschli, Protozoa. Bronn's Thierreich, I, Abth. 1, p. 517, Leipzig and Heidelberg, 1882. Wasielewski, Sporozoenkunde, 1896, p. 51.

and picro-acid fuchsin. The cell nucleus becomes more and more flattened, owing to the pressure of the parasite, and is displaced to one side (Plate XXII, Figs. 7, 10, 11, and Plate XXIII, Figs. 13, 14). Sometimes it is moulded cup-like upon the surface of the sporont (Plate XXII, Fig 7, infected cell on the right); sometimes it appears shrunken or entirely flattened by the pressure of the sporont (Plate XXII, Fig. 7, infected cell on the left).

The nucleus of the young sporont is generally eccentric in position (Plate XXII, Figs. 3, 7) and contains one or two highly-refractive, deeply-staining karyosomes. Usually at this stage and later a clear, non-staining area (karyolymph) surrounds the karyosome and the chromatic mass within or on the edge of which the karyosome lies. The nucleus may undergo a precocious division and the daughter-nuclei distribute themselves near the periphery of the sporont (Plate XXII, Fig. 5). This arrangement is seen much more clearly in the mother-sporoblast stage (Plate XXII, Figs. 6 and 7), and is obviously the precursor of the formation of daughter-sporoblasts.

2. *The formation of spores (stage of mother-sporoblast and sporoblast).*—In the mother-sporoblast stage the parasite has increased much in size, measuring 40 μ in diameter in the fresh condition. At this stage there are sometimes 2 to 4 karyosomes in each nucleus, indicating a precocious segmentation of chromatic material preparatory to the formation of sporozoites (Plate XXII, Figs. 6 and 7).

The nucleus is now in the most favorable condition for study, but on account of its minuteness we have learned very little about it. Figs. 8 and 9 (Plate XXII) represent the usual conditions met with. The nucleus may be defined as a vesicle filled with clear fluid within which are the chromatin mass and one or sometimes two karyosomes. Sometimes karyosomes only are seen (Plate XXII, Fig. 9). When there are two, one is usually much smaller than the other, and stains darker. Not the slightest indication of mitotic phenomena has been seen. The vesicular structure of the nucleus is not retained apparently beyond the mother-sporoblast stage. In all later stages we have found merely one or more round, intensely-staining bodies to all appearances identical with the karyosomes of earlier stages.

The mother-sporoblast stage is marked by a migration of the nuclei to the periphery. They are usually spaced more evenly than Fig. 6 (Plate XXII) would seem to indicate. At this stage there are often 2 to 4 karyosomes in each nucleus (Plate XXII, Figs. 6 and 7). The cytoplasm surrounding each nucleus begins to be segmented off from the rest and forms a boss on the surface of the mother-sporoblast (Plate XXII, Fig. 10). For a brief period the daughter-sporoblasts are arranged in the form of a rosette (Fig. 1, lower left-hand corner). This is due to the centrifugal mode of development of the daughter-sporoblasts, as clearly shown in the figures just mentioned. They appear to bud out from the central mass, now entirely devoid of nuclei, and then rapidly to resorb the greater portion of the mass in question while their peduncles of attachment become reduced. One or two residual masses of angular form are often seen at a later stage, wedged in among the brood of daughter-sporoblasts. In other cases there appears to be no restiform body and our conclusion is that it has been entirely resorbed by the daughter-sporoblasts. The latter, to the number of 6 to 14, rarely more, are soon entirely separate and free within the vacuole (Plate XXIII, Figs. 13 and 14). They are nearly spherical in shape and average 12 μ in diameter, although smaller and possibly abortive ones are often found in the vacuole or cyst.

At this stage the host cell still maintains its attachment to the basement membrane by means of the much-attenuated peduncle. It nearly occludes the lumen of the tubule (Plate XXIII, Fig. 13). Its cytoplasm has been reduced to a mere shell enclosing the vacuole. The nucleus still persists.

3. *The formation of sporozoites.*—The nucleus of each daughter-sporoblast undergoes repeated divisions and these nuclear divisions are foreshadowed by divisions of the karyosomes at a stage considerably in advance of the formation of the sporoblasts themselves. These precocious divisions as well as those which come after the formation of the daughter-sporoblasts (Plate XXIII, Fig. 15) are preparatory to the elaboration of the sporozoites. These, to the number of 30 to 35, are so packed in the spore that most of them lie with their

long axes in one direction. The short axis of the spore is parallel to the long axis of the sporozoites (Plate XXIII, Figs. 16 and 17). In the fresh condition a ripe spore averages 16μ by 13μ. A transverse optical section (Plate XXIII, Fig. 18) shows that the sporozoites occupy very nearly all the space within the cyst. At an earlier stage than that represented in Fig. 18 a restiform body is seen within the spore. Before the spores are fully formed they may be seen attached to this body by one extremity (Plate XXIII, Fig. 17). It is probably entirely resorbed by the sporozoites.

The sporozoites are rather strongly curved and are blunt at both ends (Plate XXIII, Fig. 19). They measure 7 μ in a straight line from tip to tip and 3 μ in thickness. The nucleus is located midway of the length and is frequently elongated (Plate XXIII, Fig. 16). In the fresh state the sporozoite exhibits a number of granules, one of larger size than the rest often being seen at one extremity (Plate XXIII, Fig. 19). The living spores are always translucent enough to enable one to see the sporozoites within. This is not invariably true of spores in sectional material stained with ferric-alum hæmatoxylin. This stain is readily extracted from the cyst-walls of a certain number of the spores, but is retained by others to such a degree that they appear perfectly black (Plate XXI, Fig. 2 and Plate XXIII, Fig. 16). This difference we believe due to a slight increase in thickness or imperviousness of the cyst wall.

The spore membrane appears to be entirely structureless. In optical section, even with very high powers, it appears merely as a sharp line. Strong artificial digesting fluids (peptic and pancreatic) do not entirely dissolve it. It is not difficult to rupture it by cover-glass pressure and set free the sporozoites.

B. THE GLOMERULAR PARASITE.

In many of the glomeruli of certain heavily-infected kidneys we have seen a parasite of considerably larger dimensions and irregular lobate form (Plate XXI, Fig. 1, lower right corner, and Plate XXIII, Fig. 20). Its habitat is the thin epithelium—usually the visceral layer—of Bowman's capsule. It encroaches inward strongly on the glomerulus.

Only a few stages in the development have been seen. That represented in Fig. 20 (Plate XXIII) is perhaps the commonest. Every gradation between this and the later stage shown in Fig. 21 has been found, but earlier stages are extremely rare. After prolonged search we have found only one example of what corresponds to the very young or sporont stage of the tubule parasite. This had a large, oval, strongly-stained nucleus with a single karyosome. The parasite was embedded by a little more than half its diameter in a much-thickened region of Bowman's capsule close to the neck. As we have seen no intermediate stages between this and that shown in Fig. 20, we cannot positively assert that these two are genetically connected. Between the stages represented in Figs. 20 and 21 every intermediate condition has been seen. Masses of protoplasm are segmented off around each one of the numerous nuclei and each mass becomes eventually transformed into a falciform body. These are somewhat variable in size and shape, as shown in Fig. 21. On account of this variability it is difficult to state their dimensions. Perhaps 7 μ by 2 μ is a fair average.

How shall the glomerular body be interpreted? While we have failed to prove even that it belongs to the same species as the parasite in the convoluted tubules, there is very strong presumptive evidence that it does, inasmuch as we have not found it in any kidney not infected with *Klossiella muris*. On the other hand, we have failed to find it in certain lightly-infected kidneys.

The glomerular parasite may be brought within the cycle of *Klossiella muris* in one of two distinct rôles. In the first place, it may represent the schizogonic or Eimerian cycle of the species, and as such may antedate the whole sporogonic cycle of the tubules. If this be true the falciform bodies shown in Fig. 21 represent the merozoites, and the infection of the tubular epithelium is due entirely to the passage of these with the secretion along the tubule until a suitable host cell is found, into which the merozoite penetrates.

Another interpretation would regard this as the male element (mother-microgametocyte), in which the falciform bodies represent the microgametocytes and from the latter the microgametes may

arise somewhat as described by Siedlecki[5] for *Adelea ovata*, i. e. after the microgametocyte has reached the macrogamete. In one instance we have seen a falciform, nucleated body adherent to a young sporont, and in two or three instances, in fresh material, a granular-looking body in the same position. As we have not seen any flagellated bodies or the penetration of a nucleus into the female element, we lack the necessary evidence that the adherent body in question is actually the male element.

C. THE PROBABLE LIFE-CYCLE OF *Klossiella muris* ACCORDING TO THE PRECEDING INVESTIGATIONS.

We have suggested two quite divergent interpretations of the glomerular parasite neither of which is in conflict with our present knowledge of Coccidia. If we interpret this body as the male element its position in the capsular epithelium is almost a necessary one for it to occupy in order that the microgametocytes may reach the tubular epithelium. If we accept the interpretation that the glomerular parasite represents the asexual or so-called Eimerian cycle which furnishes the means for an internal multiplication, or, pathologically speaking, an auto-infection of limited extent, the position of the body in the glomerulus is still a prerequisite for the success of the next stage, the invasion of the tubular epithelium by the resulting merozoites. The final interpretation probably will not be made until analogous forms in other species of coccidia shall have been discovered in which the significance of the two stages may be subjected to demonstration. This species seems to be the first one in which two forms, genetically related, occupy epithelial cells of somewhat different morphological and physiological characters.

The spores, as already stated, pass out in the urine and are most likely taken in by mice in the food and water. Direct infection is inferred from the large percentage of infected mice which are found in cages where numbers have been living together for several months at least. This observation frequently made by one of us in the study

[5] *Annales de l'Institut Pasteur*, 1899, xiii, p. 169.

of sarcosporidiosis led to a number of feeding experiments with infected kidneys. The method of feeding and of keeping the mice under observation was the same as that used in the study of *Sarcocystis muris*, to which the reader is referred for details.[6] Only kidneys containing spores with fully-developed sporozoites were used. These were finely divided and mixed with crumbs of bread softened in physiological salt solution. The dish containing this food was placed in the jar with the mouse or mice to be infected. The limited number of experiments furnished no conclusive evidence. Only one deserves mention.

A mouse fed December 18, 1900, was killed February 16, 1901. The kidneys, carefully examined fresh and in sections, contained young stages only. In the spontaneous cases early and advanced stages occur together, suggesting repeated infection. In this the occurrence of only one stage is what we should expect from a single infection.

The falciform bodies (sporozoites) set free in the digestive tract probably bore their way into the circulation which carries them into all parts of the body. Their minute size permits them to enter the smallest capillaries. In the kidneys they leave the capillaries in the glomerulus to invade the capsular epithelium and the epithelium of the convoluted tubules. In all other organs they are probably suppressed.

PATHOGENESIS.

The presence of cell-parasites like the one under consideration is evidently not indifferent to the organ in which they multiply and mature; and it remains for us to consider very briefly what injuries may be inflicted and what processes induced.

The youngest stage of the parasite (sporont) leads to the formation of a vacuole in the epithelial cell which increases with the growth of the parasite. Its very attenuated walls may still be seen after the formation of the sporoblasts. As the sporont enlarges into the mother-sporoblast and from this about 12 spores are segmented off, each larger than the original sporont as first seen within the cell, the

[6] *Journal of Experimental Medicine*, 1901, vi, p. 1.

tubule within which the parasite is lodged becomes compressed, especially when a number of the parasites have matured close together. The compression causes the adjacent epithelium to disappear, and the tubule looks like an elongated contorted bag filled with spores but largely devoid of epithelium.

No so-called inflammatory reaction can be associated with these stages. The process is a mechanical one, leading to cell destruction. There is, however, another phenomenon associated with extensive invasion of the kidneys. These organs show over the entire surface a large number of minute grayish spots, already referred to. These minute spots represent foci of necrobiotic changes in the cortex, followed by marked cell proliferation in the same areas. The less advanced foci are largely made up of the tubular epithelium of the area which has become, as it were, fused into a mass on account of the obliteration of the lumina of the tubules. In later stages the original tissue becomes gradually obscured by the active proliferation of the intertubular tissue, giving rise to a dense collection of cell nuclei with scant cytoplasm. These foci resemble early foci in tuberculosis when giant cells are absent. The cells themselves correspond to what are usually called round cells. They often appear sheathing the larger blood-vessels of the cortex. The genesis of these foci is obscure, but they are probably due to *Klossiella muris*, as we have not seen them excepting in the presence of this parasite. It is probable that they represent older foci of invasion which had been abandoned by the ripe spores, and the devastated tubules have become the seat of an interstitial process which in its progress may envelope and obliterate neighboring normal tubules as well. Processes associated with acute inflammatory reactions were not encountered. Polynuclear leucocytes as well as exudations were absent.

SYSTEMATIC POSITION OF THE RENAL PARASITE.

The classification of the Coccidia has undergone more or less change in recent years. The last attempt by Schaudinn[7] following Léger is perhaps the best. Of the 3 families there recognized, *Disporo-*

[7] Untersuchungen üb. d. Generationswechsel bei Coccidien. *Zool. Jahrbuch., Abth. f. Morphol.*, 1900, xiii, p. 197.

cystidea, *Tetrasporocystidea*, and *Polysporocystidea*, the mouse parasite clearly belongs to the third. When we come to the genera our difficulty begins, for these are based on the contents of the individual spore. Sporocysts with 1, 2, 3 and 4 sporozoites are accounted for, but our parasite contains about 30 sporozoites. We have therefore preferred to create a new genus rather than enlarge the scope of any of the existing genera for its reception. We have named the new genus *Klossiella*,[8] of which the type and only known species is *Klossiella muris*. The following is offered as a diagnostic description of the same:

Klossiella muris, gen. et spec. nov. Sporogonic cycle characterized by the development of 12 to 14 spheroidal spores, measuring 16 μ by 13 μ, each of which contains 30 to 34 banana-shaped sporozoites.

Schizogonic cycle unknown, but a phase of unknown significance in the glomerulus.

Sporont with 10 to 20 spherical plastin granules, 1.5 μ in diameter, and smaller granules of the same physical and chemical properties. The latter persist until sporozoite-formation.

Habitat: Renal epithelium (convoluted tubules and glomerulus) of *Mus musculus*.

DESCRIPTION OF PLATES XXI-XXIII.

(The figures on Plate XXI are reproduced from photomicrographs made with the kind permission of Dr. J. H. Wright by Mr. L. S. Brown in the Clinico-pathological Laboratory of the Massachusetts General Hospital. The figures on Plates XXII and XXIII are from camera lucida drawings made with a Leitz 1/12 homogeneous immersion objective. The fixing agent in all cases was Zenker's fluid.)

PLATE XXI.

Fig. 1. Photomicrograph of an area in a section of the cortex of a heavily-infected kidney, showing *Klossiella muris* in the mother- and daughter-sporoblast stages. In the lower right-hand corner is a portion of a glomerulus, showing two parasites in different stages in the epithelium of Bowman's capsule. × 750.

Fig. 2. Photomicrograph of an area of the cortex of the same kidney, showing sporocysts (spores). Some have resisted decolorization and appear

[8] We are indebted to Dr. C. W. Stiles for suggesting to us the generic name *Klossiella* in place of the less accurate Microklossia which we had selected.

as black balls. In others the nuclei of the sporozoites can be made out distinctly. × 800. (Both stained in ferric-alum hæmatoxylin.)

PLATE XXII.

Fig. 3. An infected cell from a fresh kidney, teased in salt solution and stained with aceto-methyl green. It contains a sporont within a large vacuole. The spherical refractive bodies in the sporont are plastin granules. × 850.

Fig. 4. A sporont containing both large and small granules. In salt solution; unstained. × 850.

Fig. 5. A double infection. The nucleus has already divided and become distributed towards the periphery. Hæmalum. × 737.

Fig. 6. Mother-sporoblast, showing nuclei near periphery, containing one to four karyosomes and a mass of granular, less intensely-staining chromatin. Hæmalum. × 1435.

Fig. 7. Longitudinal section of a portion of a convoluted tubule, with three infected cells almost occluding its lumen. The two cells to the right show the attachment of the infected cell by a narrow densely-granular peduncle to the basement membrane. The increase in size of the infected cells is clearly seen. Hæmalum and picro-acid fuchsin. × 870.

Figs. 8 and 9. Nuclei of mother-sporoblast stage. × 1700.

Fig. 10. Mother-sporoblast, with daughter-sporoblasts beginning to form. Shrinkage during fixation is probably responsible for the protrusion of one sporoblast beyond the normal contour. Hæmalum. × 1475.

Fig. 11. A stage a little later than the preceding. The daughter-sporoblasts, each with its nucleus at its distal end, appear to bud out from a large central mass. Ferric-alum hæmatoxylin. × 1590.

Fig. 12. The daughter-sporoblasts nearly complete, still attached by a narrow peduncle to central restiform body. Ferric-alum hæmatoxylin. × 1590.

PLATE XXIII.

Fig. 13. Transverse section of convoluted tubule, with very large flask-shaped cell, containing *Klossiella* in the daughter-sporoblast stage. The infected cell nearly fills the lumen. It still retains its attachment to basement membrane by a narrow peduncle. Hæmalum; picro-acid fuchsin. × 870.

Fig. 14. Infected cell from fresh kidney, in salt solution. It contains 14 sporoblasts in which the small plastin granules are conspicuous. The cell is more or less flattened by pressure of the cover-glass. × 850.

Fig. 15. Small brood of sporoblasts, showing division and distribution of nuclei preparatory to formation of sporozoites. Hæmalum; picro-acid fuchsin. × 870.

Fig. 16. Brood of spores still within cell-membrane of the completely atrophied cell. Six spores show the sporozoites within the cyst-wall; four are solidly stained and opaque. Ferric-alum hæmatoxylin. × 870.

Fig. 17. Spore from fresh kidney, in salt solution, showing sporozoites still attached to restiform body. × 850.

Fig. 18. More advanced spore, fresh. Aceto-methyl green. Transverse optical section. The nucleus appears in four sporozoites. × 850.

Fig. 19. *a*, *b*, two sporozoites in fresh condition, liberated from spore cyst by crushing; *c*, sporozoite from a spore softened by peptic digestion. × 850.

Fig. 20. Section of glomerulus, showing glomerular parasite *in situ* in visceral layer of Bowman's capsule. × 485.

Fig. 21. Falciform bodies, oldest stage of glomerular parasite observed. Hæmalum, picro-acid fuchsin. × 1590.

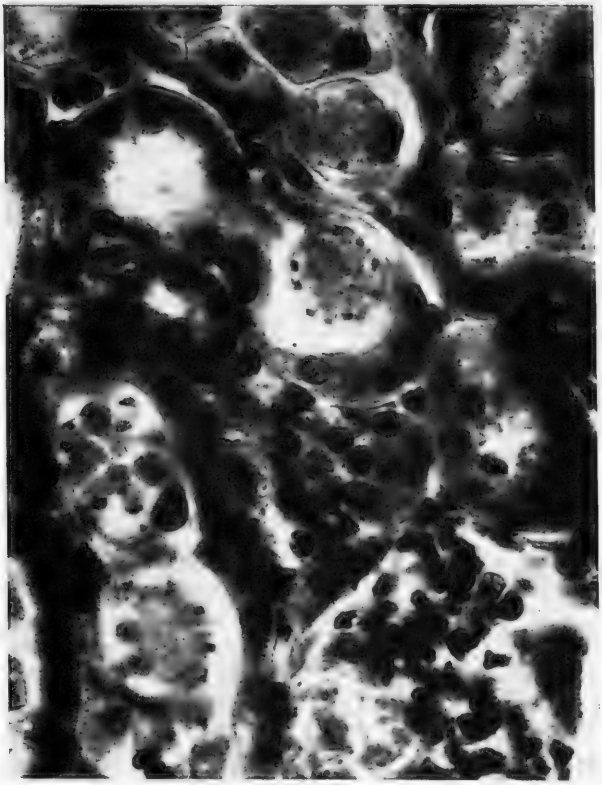
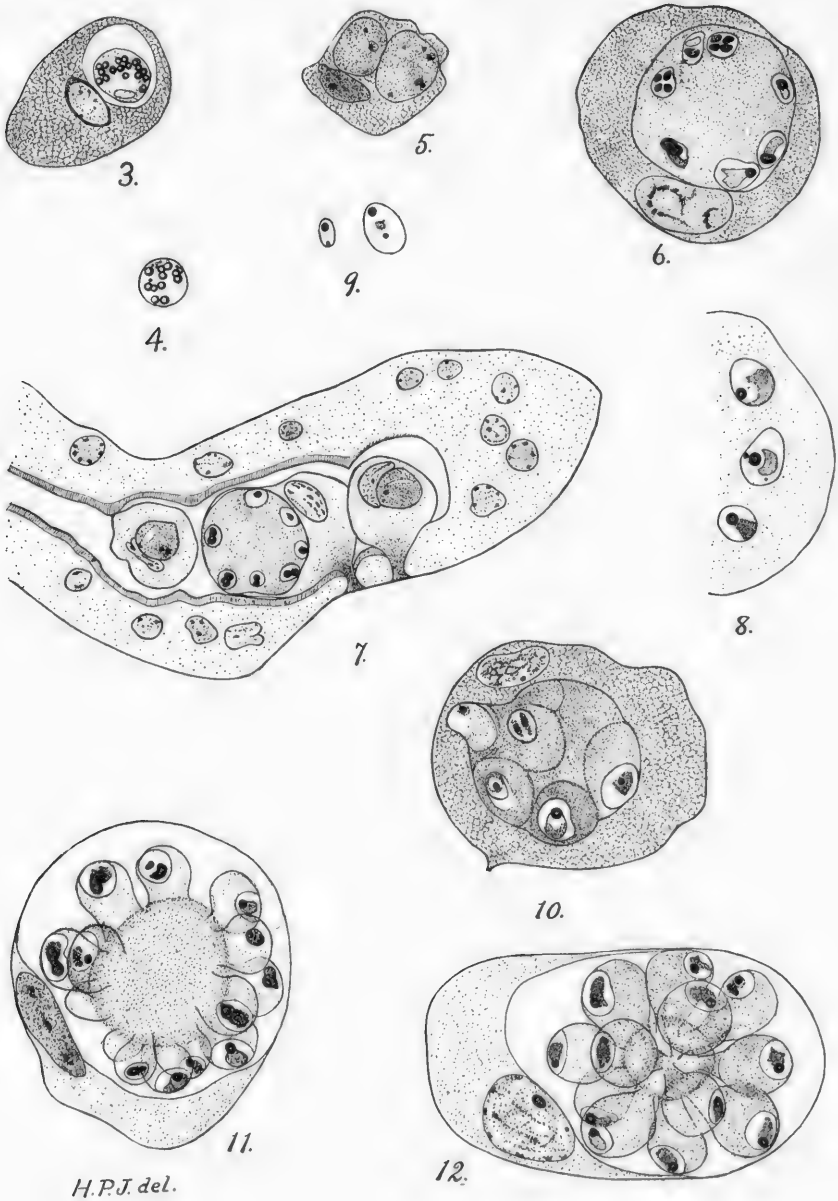


FIG 1.

FIG. 2.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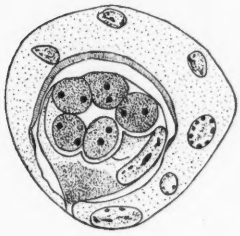

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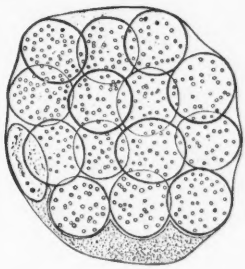

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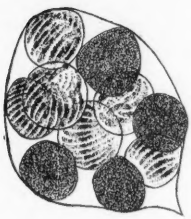

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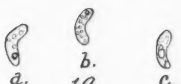
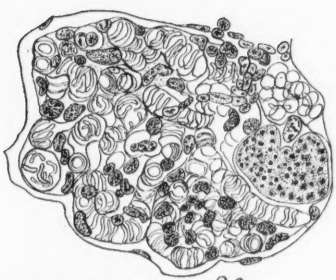
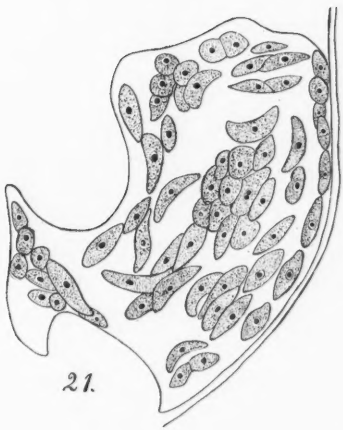


a. b. 19. c.



H.P.J. del. 20.



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